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Influence of fishmeal-replaced diet on nutrient digestibility, digestive enzyme activity, and whole-body fatty acid profile of Indian major carp, *Cirrhinus mrigala*

Chiranjiv Pradhan[1,2*], Shiba Shankar Giri[1], Satyandra Nath Mohanty[1] and Khiroda Chandra Nayak[1]

Abstract

Background: The aquaculture sector is the biggest consumer of fishmeal and the demand of this ingredient is higher than the production. The replacement of fishmeal from aquafeed is a current research thrust. The replacement of fishmeal has been possible with plant ingredients in many fish species, however, possesses some limitations in terms of low digestibility and inadequate nutrient profile.

Results: Complete replacement of fishmeal in the diets of *Cirrihinus mrigala* was studied using specially designed nutrient digestibility system. Two feeds were prepared, one with fishmeal as one of the ingredients (control feed) and another with complete plant ingredients (test feed). Initially, forty *C. mrigala* fingerlings with average weight of 10.88 ± 0.17 g were stocked in six tanks. Fish were fed up to satiation twice daily (10:00 and 16:30 h) for a period of 45 days. After 1 week of acclimatization, fecal samples were collected for 45 days. At the end of the experiment, no significant differences ($P > 0.05$) were observed for somatic and serum parameters in two treatments. The digestibility coefficient of dry matter, protein, lipid and energy, and three digestive enzymes; amylase, proteases, and lipase did not differ significantly ($P > 0.05$) between the treatments. The whole body proximate compositions were similar between the treatments. The test diet (containing linseed oil) fed group exhibited higher proportions of whole body linolenic acid (ALA, 18:3n-3). However, eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) did not vary in both studied treatments.

Conclusion: The information obtained from this study revealed that fishmeal can be completely replaced from the diet of *C. mrigala* without affecting their growth and nutrient digestibility and fatty acid composition.

Keywords: Fishmeal replacement, Carp, Nutrient digestibility, Digestive enzymes, Fatty acid profile

Background

Aquafeed industry is heavily dependent on fishmeal (FM) due to the nutritional quality and high palatability. The production of FM is almost static over the last decade whereas the demand is huge (Tacon & Metian, 2015). The price of this ingredient is increasing continuously thus substitution of FM by alternate protein sources from aquafeeds becomes a research priority. There are numerous protein sources such as animal by products, oil meals, insect meals, and single-cell proteins which can be used as potential substitutes of FM in aquafeeds (Hua et al., 2019). Terrestrial plant protein ingredients such as soybean meal, rapeseed meal, groundnut oil cake, and corn gluten meal have been used as suitable alternatives of FM in the diets of fish because of their suitable nutritional properties and high global availability at competitive prices.

* Correspondence: cpradhankufos@gmail.com
[1]ICAR-Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar, Odisha 751002, India
[2]Department of Aquaculture, Kerala University of Fisheries and Ocean Studies, Panangad, Kochi 682506, India

A number of studies were conducted to replace FM with plant ingredients in several fish species which showed restricted feed intake, low growth rate, less protein utilization, and caused physiological imbalances due to less palatability, inadequate nutrient profile in terms of amino acid, fiber content, and presence of anti-nutritional factors (Francis, Makkar, & Becker, 2001; Pratoomyot, Bendiksen, Bell, & Tocher, 2010; Turchini, Torstensen, & Ng, 2009). The anti-nutritional factors contribute to the poor digestion by altering the activities of digestive enzymes and absorption of the nutrients by different interactive processes. However, replacement of a large proportion of dietary FM or complete replacement with a suitable blend of plant protein has been possible in many carnivorous, omnivorous, and herbivorous fish species such as salmon (Crampton, Nanton, Ruohonen, Skjervold, & El-Mowafi, 2010), tilapia (Thobaitia, Ghanima, Ahmeda, Sulimana, & Mahboob, 2018), and common carp (Adekoya, Porcadilla, Varga, & Kucska, 2018). It is of paramount importance to study the effect of plant ingredients at different combinations in a diet on growth, nutrient utilization, digestive physiology, and health status of different fish species.

Growth is the important expression of fulfillment of nutrient requirement and followed by nutrient utilization in fish. A suitable blend of plant ingredients compensates their nutrient deficiency and leads to a better nutrient profile of feed which on utilization is translated into productivity. The nutrient utilization is best assessed from the apparent digestibility coefficients (ADC) of nutrients and energy in feedstuffs which also helps for correct diet formulation. The ability of fish to metabolize a diet depends on the physical and chemical nature of the food and the availability of appropriate digestive enzymes, which mediate specific degradation pathways for maximum utilization of nutrients (Phillips, 1969). The capacity of digestion for certain food materials by an animal is predominately dependent on the presence of appropriate enzymes (Weinrauch, Schaefer, & Goss, 2019). The fish reared with fishmeal replaced diet with plant ingredients may develop some unhealthy condition which can be easily monitored through blood biochemical analysis. The blood biochemical studies always help in the assessment of alteration in the physiological state of fish exposed to any change in the feed, feeding, and habitat (Acharya & Mohanty, 2019; Deng et al., 2017). There are also many reports on blood metabolic responses to inclusion of different plant ingredients in the diet of carp (Kriton et al., 2018; Pradhan, Giri, Mohanty, & Mohanty, 2020).

There are several benefits of long-chain polyunsaturated fatty acids (PUFA) for the normal growth and health status of the fish (Misra et al., 2006). However, there is a paucity of information on the role of long-chain PUFAs such as eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic (DHA, 22:6 n-3) on the growth performance of carps. Similarly, studies related to the use of vegetable oil rich in n-3 fatty acid such as alpha-linolenic acid (ALA, 18:3 n-3) to check the conversion efficiencies in carps are also limited. Supplementation of linseed oil against the residual fish oil from FM in a mixture of plant protein ingredients may be an ideal strategy to improve the fatty acid profile of *C. mrigala*.

In the present study, the effects of complete substitution of FM by plant ingredient mixtures in *C. mrigala* diets were evaluated through studying their growth performance, feed utilization, nutrient digestibility, digestive enzyme activity, blood biochemical changes, carcass composition, and whole-body fatty acid profiles.

Methods

Experimental diets

Two feeds were prepared, one with fish meal (Control feed) and then another one without FM only with plant ingredients (test feed) (Table 1). All the ingredients were procured from Central Institute of Freshwater Aquaculture (CIFA) stores. In control feed, fishmeal was incorporated, which was absent in the test feed. Similarly, mustard oil cake and linseed oil were added in the test feed, which were absent in the control feed. The ingredients were grounded into powder and mixed. The feed mixture was fortified with vitamin-mineral mixture and oil and pelletized in semi-industrial motorized pelletizer. The dried extruded pellets were then crumbled to adequate size to fit into the mouth of the fish fingerlings, packed in airtight plastic bags, and used for feeding. The two feeds were iso-nitrogenous and iso-lipidic. The fatty acid profiles for the experimental diets expressed as percentage of total fatty acids. Total saturated fatty acids were 24.61% and 21.25% in the control and test feed, respectively. Total monounsaturated fatty acids (mainly oleic acid, 18:1 n-9) were 43.49% in the control feed and 42.29% in the test feed. Alpha-linolenic acid (18:3 n-3) was 0.81 and 3.70% in control and test feed, respectively. There was no EPA and DHA present in the test feed.

Fish, experimental system, and feeding

The Indian major carp, *C. mrigala* fingerling were brought from CIFA farm and stocked in the FRP tanks. Before stocking to the experimental tanks, the fish were treated with potassium permanganate ($KMnO_4$) solution (1 ppm) to remove the ectoparasite, if any. Forty *C. mrigala* fingerlings were stocked in each tank with an initial mean weight of 10.88 ± 0.17 g. The experimental tanks were specially designed cylindro-conical FRP tanks of 200 L capacity. The tanks were equipped with a valve that controls water passage into decanting reservoirs fitted to the bottom of the tank for the collection of feces

Table 1 Dietary formulation (g kg^{-1}) and chemical analysis (g kg^{-1} dry matter) of the experimental diet

Feed ingredients	Dietary treatments	
	Control feed	Test feed
Soya bean meal	200.0	299.1
Groundnut oil cake	350.0	141.5
Mustard oil cake	0.00	236.4
Rice bran	380.0	293.0
Fish meal	50.0	0.00
Linseed oil	0.00	10.0
Vitamin[a] and mineral[b] Premix	20.0	20.0
Chemical composition		
Crude protein	307.4	306.2
Crude lipid	72.4	67.8
Ash	117.5	122.7
Fiber	98.4	101.4
NFE[C]	404.3	401.9
Total energy ($MJkg^{-1}$)	17.12	16.92
Fatty acids		
ΣSFA[d]	24.61	21.25
ΣMUFA[e]	44.82	42.29
ΣPUFA[f] n-6	27.16	27.40
18:3 n-3	0.81	3.70
20:5 n-3	0.15	ND
22:6 n-3	0.39	ND
ΣPUFA n-3	1.35	3.70

[a]Vitamin (IU or g kg^{-1} premix): retinol palmitate, 50,000 I.U; thiamine, 5; riboflavin, 5; niacin, 25; folic acid, 1; pyridoxine, 5; cyanocobalomin, 5; ascorbic acid, 10; cholecalciferol, 50,000 IU; α-tocopherol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; choline chloride, 100; biotin, 0.25
[b]Minerals (g kg^{-1}): CaCO3, 336; KH2PO4, 502; MgSO4.7H2O, 162; NaCl, 49.8; Fe(II) gluconate, 10.9; MnSO4.H2O, 3.12; ZnSO4.7H2O, 4.67; CuSO4.5H2O, 0.62; KI, 0.16; CoCl2.6H2O, 0.08; ammonium molybdate, 0.06; NaSeO3, 0.02
[C]Nitrogen free extract (NFE): 1000- (protein + lipid + fiber + ash)
[d]*SFA* saturated fatty acid
[e]*MUFA* mono unsaturated fatty acid
[f]*PUFA* poly unsaturated fatty acid

and uneaten food materials. Each tank was plumbed with a water recirculation system with a water flow rate of 3 lit/min. The aerated water was pumped into the biofilter system and then to the overhead tank for circulation into the tank. Three tanks were allocated under each dietary treatment. The fish were acclimated to these tanks and the experimental diets (control and test feed, Table 1) for 1 week. Triplicate groups of fish were fed till satiation, twice daily, at 10:00 and 16:30 h for 45 days. The daily feed supplied was recorded and uneaten feed was collected after 30 min of feeding. The fecal matter was collected daily morning in a plastic container and stored in −80 °C for further analysis. The nutrient content of fecal matter was analyzed for nutrient digestibility following the direct digestibility method. During the experimental period, water temperature was 28.55 °C, pH 7.4; DO 7.08 mg/l, the total alkalinity 72 mg/l; ammonia nitrogen 0.02 mg/l; nitrite nitrogen was less than 0.01 mg/l. The nitrate-nitrogen 0.02 mg/l and phosphorous were less than 0.01 mg/l in the experimental tanks.

Sampling

At the end of the trial, the fish were not supplied with feed and allowed to starve for 24 h, counted and individually weighed to determine survival rate, weight gain (WG), specific growth rate (SGR), and feed conversion ratio (FCR). Fifteen fish were randomly sampled from each tank. Five fish were frozen at −20 °C for whole-body proximate composition determination, five fish for digestive enzymes, and from the rest five fish, blood samples were collected from caudal venifuncture, killed, and preserved at −80 °C for fatty acid analysis. The fish were kept for digestive enzyme analysis dissected to obtain liver and viscera. Viscerosomatic index (VSI) and hepatosomatic index (HSI) were also calculated. The intestine contents were smoothly removed; 10 cm of the anterior intestine (AI) was cut and rinsed with ice-cold saline solution. The tissue was homogenized in 50 mM cold phosphate buffer (pH 7.0) in an ultrasonicator at ratios of 1:10 (tissue: buffer) and the homogenate was centrifuged at 5000×*g* for 20 min. All the operations were carried out at 4 °C. The supernatant containing the enzymes was stored at −20 °C until analyzed. The blood sample were collected both with addition of heparin and without anticoagulant. Serum was separated from the coagulated blood by centrifugation at 5000 rpm for 5 min at 4 °Cand thereafter stored at −20 °C for further analysis.

Proximate analysis

The moisture, crude protein, crude lipid, and ash were analyzed using standard methods (AOAC, 1998). The moisture was determined by oven drying to constant weight at 110 °C for 24 h. However, the moisture content of fecal matter was removed using lyphilizer. Crude protein content was determined by Kjeldahl analysis (nitrogen × 6.25; Kjeltec Autoanalyzer, Vapodest-50, Gerhardt, Germany). Crude fat was determined by Soxhlet extraction using petroleum ether (60–80 °C boiling point) on a Soxtec System (Pelican instruments, Chennai, India). The ash content was determined by igniting a porcelain crucible in a muffle furnace at 550 °C for 4 h. Gross energy of the feeds was determined using a bomb calorimeter (IKA C5003 control, Japan).

Fatty acid analysis

Feed and flesh were homogenized in (2:1 v/v) chloroform-methanol mixture containing 0.01% BHT with a mechanical

stirrer. Total lipids were prepared as per the method described by Folch, Leeds, and Sloane-Stanley (1957). The weight of lipids was determined gravimetrically after evaporation of the solvent. Fatty acid methyl esters (FAME) were prepared by acid-catalyzed transesterification of total lipids according to the method of Christie (1982). Fatty acid methyl esters were separated by a gas chromatograph equipped with a flame ionization detector (Shimadzu GC-2010, Kyoto, Japan) on a DB-25 capillary column (20 m × 0.10 mm I.D., 0.10 μm, J&W Scientific, Santa Clara, CA, USA). The detailed separation method was followed as per Pradhan, Giri, Mohanty, and Narasimmalu (2019). The fatty acids were identified using FAME standards. Area percentage normalized values for the fatty acids were taken as weight percentages.

Analysis of digestive enzymes

Three digestive enzymes such as protease, amylase, and lipase were determined. Protease activity of the intestine was measured following the casein digestion method described by Kunitz (1947). The reaction mixture consisted of 1% casein as substrate (0.25 mL), phosphate buffer (pH 7.0) (1 ml), and tissue homogenate (0.25 ml) was incubated at 37 °C for 2 h. The reaction was stopped by adding 10% trichloroacetic acid (TCA), kept for overnight at 4 °C, centrifuged for 10 min, and the supernatant was collected. The protein concentration of supernatant was measured at 600 nm following Lowry, Rosebrough, Farr, and Randall (1951). The activity of protease enzyme was determined as microgram hydrolyzed proteins released per min per milliliter sample.

Amylase activity was assayed with 1% (w/v) starch solution as substrate in 0.05 M phosphate buffer. A total of 0.5 ml of substrate solution was added to 0.25 ml of enzyme preparation. The reaction mixture was incubated at room temperature for 15 min. Dinitrosalicylic acid (DNS) was added and kept in a boiling water bath for 10 min. Forty percent sodium-potassium tartrate was added to stop the reaction. After cooling, the reaction mixture was diluted with distilled water and absorbance was recorded at 575 nm. Amylase activity was determined from the glucose standard curve and expressed as microgram reducing sugars released per min per milliliter (Bergmeyer, 1974).

Lipase activity was determined following Love (1986), with the principle that when the substrate 1,2-O-dilauryl-rac-glyero-3-glutaric acid-(6′methyl-resorufin)-ester is cleaved by pancreatic lipase and the resulting carboxylic acid ester is hyrolyzed under the alkaline test conditions to yield the chromophoremethylresorifine. The kinetic of color formation at 580 nm is monitored and it is proportional to lipase activity in sample. It is represented in the unit u/l.

Analysis of blood metabolites

The heparinated blood samples were analyzed for hemoglobin concentration by the cyanmethemoglobin method. The serum was used to determine the glucose by enzymatic methods. Total serum protein, albumin, cholesterol, triglycerides, HDL, and LDL were determined from serum by using a commercial kit (Chema diagnostic, Mansano (AN) Italy-EU). Globulin was determined by deducting albumin value from protein value and VLDL was determined from the fifth fraction of triglyceride values. All samples were analyzed in duplicates following the manufacturer's instructions.

Calculations and statistical analysis

The weight gain, specific growth rate (SGR), viscera-somatic index (VSI), hepato-somatic index (HSI), survival percentage, and protein efficiency ratio (PER) were determined from the following formula:

$$\text{Weight gain (g)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{SGR} = 100 \times [\ \ln(\text{Final weight (g)}) - \ln(\text{Initial weight (g)})] \times \text{Days of experiment}^{-1}$$

$$\text{Hepato - somatic index (HSI)} = 100 \times [\text{Weight of liver (g)}] \times [\text{Weight of fish (g)}]^{-1}$$

$$\text{Viscero - somatic index (VSI)} = 100 \times [\text{Weight of viscera (g)}] \times [\text{Weight of fish (g)}]^{-1}$$

$$\text{Survival}\% = (\text{Number of fish on final harvest} \times \text{x Number of fish at initial stocking} - 1) \times \text{x100}$$

$$\text{Feed conversion ratio (FCR)} = \text{Feed intake (g)} \times \text{Wet weight gain (kg)}^{-1}$$

$$\text{Protein efficiency ratio (PER)} = \text{Weight gain (kg)} \times \text{Crude protein fed (kg)} - 1$$

The apparent digestibility coefficients (ADCs) of the nutrients were measured using the direct method and can be described as:

$$\text{ADC} = 100 - [100 \times (\%\text{of nutrient voided in faeces}) \times (\%\text{of nutrient ingested in feed}) - 1]$$

All the data were represented in average ± standard error. Data were analyzed through students T test using the SPSS (SPSS 16.0).

Results

Growth

The final average weight showed around 4 g increment over the initial weight. The final average weight and weight gain of *C. mrigala* were alike between the

treatments and no significant difference ($P > 0.05$) was observed. The group of fish received both fishmeal and plant ingredient-based diets showed similar feed conversion ratio (FCR). The SGR, VSI, HSI, and PER between the treatments also did not exhibit any difference (Table 2).

Nutrients digestibility

The nutrient digestibility of control and test feed in *C. mrigala* is presented in Table 3. The dry matter intake from the control feed and test feed were 260.55 and 253.72 g, respectively, and was not significantly different ($P > 0.05$) between the treatments. The fecal dry matter voided by the fish during the nutrient digestibility study also did not significantly vary from each other. The apparent digestibility coefficient (ADC) of dry matter of the control feed was 94.43% and was similar ($P > 0.05$) to that of the test feed (94.51%). The organic matter (OM) digestibility also did not vary from each other. The ADC of crude protein and crude lipid for control feed was 92.16 and 90.01%, respectively. Whereas, the ADC of crude protein and crude lipid for test feed was 90.25 and 89.12%, respectively and no significant difference ($P > 0.05$) was evident between the two feeding treatments. Nitrogen digested for control and test feed was 11.59 and 11.02 g, respectively. The percentage of nitrogen digestibility from total nitrogen intake was 90.45 and 88.76% for control and test feed respectively. Gross energy intake per day 39.98 and 37.27 kcal for control and test feed groups and was not significantly different ($P > 0.05$) from each other. The daily loss of fecal energy from the control feed group (3.38 kcal) was similar with the daily loss (3.84 kcal) of the test feed group. Total digestible energy for control feed fed group and test feed fed group per day was 36.6 kcal and 33.42 kcal, respectively. The ratio of digestible energy to gross energy between the treatments showed no significant difference.

Table 2 Growth performance of *C. mrigala* fed with fishmeal (control feed) based feed and complete vegetable ingredient (test feed) based feed

Particulars	Dietary treatments	
	Control feed	Test feed
[1]IBW (g)	10.88 ± 0.17[a]	10.88 ± 0.17[a]
[2]FBW (g)	14.66 ± 0.09[a]	14.98 ± 0.10[a]
[3]WG (g)	3.91 ± 0.33[a]	3.98 ± 0.35[a]
SGR%	0.66 ± 0.10[a]	0.71 ± 0.10[a]
FCR	1.85 ± 0.01[a]	1.77 ± 0.02[a]
PER	4.93 ± 0.46[a]	5.11 ± 0.42[a]
HSI	0.51 ± 0.02[a]	0.59 ± 0.04[a]
VSI	4.3 ± 0.2[a]	4.64 ± 0.3[a]
Survival (%)	98.6 ± 0.03[a]	98.4 ± 0.02[a]

Means of each parameter in the same row with similar superscript are not significantly different
[1]*IBW* initial body weight
[2]*FBW* final body weight
[3]*WG* weight gain

Table 3 Nutrient digestibility, nitrogen (N), and gross energy (GE) balance of experimental feed in *C. mrigala* fingerlings

Particulars	Control Feed	Test Feed
Dry matter (DM) intake (g)	260.55 ± 9.57[a]	253.72 ± 9.16[a]
Fecal DM voided	14.52 ± 0.91[a]	13.98 ± 0.88[a]
DM digested	246.04 ± 7.36[a]	239.72 ± 7.84[a]
% DM digestibility	94.43 ± 1.21[a]	94.51 ± 1.89[a]
Organic matter (OM) intake (g)	229.94 ± 4.20[a]	222.48 ± 4.77[a]
OM voided (g)	24.26 ± 1.10[a]	27.9 ± 1.61[a]
OM digested (g)	205.68 ± 5.00[a]	194.58 ± 5.37[a]
% OM digestibility	89.45 ± 2.10[a]	87.46 ± 2.90[a]
Crude protein (CP) intake (g)	80.12 ± 2.10[a]	77.65 ± 2.97[a]
CP voided in feces (g)	6.28 ± 0.94[a]	7.57 ± 0.70[a]
CP digested (g)	73.81 ± 2.06[a]	70.12 ± 2.06[a]
% CP digestibility	92.16 ± 2.05[a]	90.25 ± 2.44[a]
Crude lipid (CL) intake (g)	18.86 ± 1.26[a]	17.19 ± 1.21[a]
CL voided in feces (g)	1.89 ± 0.01[a]	1.89 ± 0.02[a]
CL digested (g)	16.977 ± 1.26[a]	15.30 ± 1.22[a]
% CL digestibility	90.01 ± 2.13[a]	89.12 ± 2.22[a]
Nitrogen (N) intake (g)	12.82 ± 0.58[a]	12.42 ± 0.55[a]
N voided in feces (g)	1.22 ± 0.01[a]	1.4 ± 0.02[a]
N digested (g)	11.59 ± 0.57[a]	11.02 ± 0.67[a]
% Nitrogen digestibility	90.45 ± 2.50[a]	88.76 ± 2.44[a]
Gross energy intake (Kcal/day)	39.98 ± 1.55[a]	37.27 ± 1.46[a]
Fecal energy loss (Kcal/day)	3.38 ± 0.07[a]	3.84 ± 0.09[a]
Digestible energy (Kcal/day)	36.6 ± 1.54[a]	33.42 ± 1.55[a]
% Digestible energy	91.54 ± 2.75	89.66 ± 3.12
DE:GE ratio	0.91 ± 0.02[a]	0.89 ± 0.02[a]

Means of each parameter in the same row with similar superscript are not significantly different

Digestive enzymes activity

The intestinal amylase activity of *C. mrigala* was 15.33 and 15.57 micromoles of maltose released min^{-1} mg^{-1} protein fed control and test feed, respectively, and did not show any significant difference ($P > 0.05$) between each other. There was no significant difference ($P > 0.05$) in the activities of protease between the treatments. The protease activity of *C. mrigala* was 43.07 and 42.55 micromoles of tyrosine released min^{-1} mg^{-1} protein in the control and test feed fed group, respectively. Similarly, the lipase activity in *C. mrigala* intestine fed control and test diet showed no significant ($P > 0.05$) difference between each other (Table 4).

Table 4 Intestinal digestive enzyme activity of *C. mrigala* fingerlings fed experimental diets

Particulars	Control feed	Test feed
Protease (micromoles of tyrosine released min^{-1} mg^{-1} protein)	43.07 ± 1.62[a]	42.55 ± 1.07[a]
Amylase (micromoles of maltose released min^{-1} mg^{-1} protein)	15.33 ± 0.61[a]	15.57 ± 0.46[a]
Lipase (U/l)	19.80 ± 1.92[a]	19.46 ± 0.99[a]

Means of each parameter in the same row with similar superscript are not significantly different

Whole-body composition of *C. mrigala* fingerlings

The whole-body proximate composition is presented in Table 5. There was no significant difference ($P > 0.05$) between the moisture content of the control and test group. The crude protein (CP) content was 17.10 and 17.19% for the control and test group, respectively. Whereas, the initial CP content of *C. mrigala* was 16.05% of body composition. The crude lipid and ash content of both control and test group were similar and did not show any statistical difference ($P > 0.05$).

Whole-body fatty acids profile of *C. mrigala* fingerlings

The whole-body fatty acid profile of *C. mrigala* fed to experimental feeds is presented in Table 6. The fatty acids C14:0, C16:0, and C18:0 were the dominated among saturated fatty acids in both studied treatments and were alike without any significant ($P > 0.05$) difference. The total saturated fatty acid (SFA) content of *C. mrigala* was 44.01 and 46.76 percentage fed control and test feed, respectively and no significant difference ($P > 0.05$) was noticed between the two treatments. The C18:1 was 75.45 and 72.5% of the total monounsaturated fatty acid (MUFA) in control and test feed groups, respectively, without any statistical difference ($P > 0.05$) between the treatments. The total MUFA contents both for control (27.19%) and test (28.00%) group were alike and did not show any significant ($P > 0.05$) difference. The polyunsaturated fatty acid (PUFA) content of both the treatments was alike ($P > 0.05$). The alpha ALA content was significantly higher in the test group. However, the EPA and DHA content of *C. mrigala* did not show any significant difference ($P > 0.05$) between each other. The sum of PUFA n-3 of the control group was not significantly different than the test group and the values were 9.29 and 10.05% for control and test feed, respectively. The ratio of saturated to PUFA and n-6 to n-3 showed no significant difference between the treatments.

Table 5 Body composition (%) of *C. mrigala* fingerlings fed experimental diets

Particulars (NS)	Beginning	End of the experiment	
		Control feed	Test feed
Moisture	74.45 ± 0.32	71.41 ± 0.19[a]	71.50 ± 0.33[a]
Protein	16.05 ± 0.12	17.10 ± 0.27[a]	17.19 ± 0.21[a]
Lipid	2.08 ± 0.08	2.21 ± 0.29[a]	2.13 ± 0.17[a]
Ash	5.09 ± 0.56	6.89 ± 0.22[a]	6.78 ± 0.30[a]

Means of each parameter in the same row with similar superscript are not significantly different

Table 6 Whole-body fatty acid (% of total fatty acids) profile of *C. mrigala* fed experimental diets

Fatty acids	Control feed	Test feed
C10:0	0.33 ± 0.02[a]	0.37 ± 0.02[a]
C12:0	0.23 ± 0.02[a]	0.18 ± 0.01[a]
C13:0	13.09 ± 0.40[a]	16.70 ± 1.35[a]
C14:0	0.83 ± 0.05[a]	0.90 ± 0.15[a]
C15:0	0.41 ± 0.02[a]	0.38 ± 0.01[a]
C16:0	15.78 ± 0.09[a]	16.15 ± 0.59[a]
C17:0	0.23 ± 0.03[a]	0.24 ± 0.00[a]
C18:0	9.53 ± 0.27[a]	7.82 ± 0.28[a]
C20:0	0.35 ± 0.04[a]	0.38 ± 0.05[a]
C21:0	ND	0.30 ± 0.02
C22:0	0.44 ± 0.02[a]	0.40 ± 0.05[a]
C23:0	2.48 ± 0.12[a]	2.54 ± 0.19[a]
C24:0	0.31 ± 0.05[a]	0.38 ± 0.02[a]
ΣSFA	**44.01 ± 0.46[a]**	46.76 ± 0.76[a]
C14:1	2.02 ± 0.96[a]	2.45 ± 0.07[a]
C15:1	0.38 ± 0.02[a]	0.39 ± 0.03[a]
C16:1	2.11 ± 0.13[a]	2.37 ± 0.24[a]
C17:1	0.28 ± 0.02[a]	0.25 ± 0.00[a]
C18:1	20.50 ± 0.24[a]	20.30 ± 1.26[a]
C20:1	0.90 ± 0.03[a]	1.39 ± 0.23[a]
C22:1	0.49 ± 0.05[a]	0.29 ± 0.04[a]
C24:1	0.51 ± 0.05[a]	0.56 ± 0.05[a]
ΣMUFA	**27.19 ± 0.93[a]**	28.00 ± 1.72[a]
C18:2n6	9.34 ± 0.10[a]	8.29 ± 0.38[a]
C18:3n6	0.43 ± 0.04[a]	0.50 ± 0.00[a]
C20:2n6	0.77 ± 0.05[a]	0.79 ± 0.04[a]
C20:3n6	5.25 ± 0.15[a]	5.01 ± 0.24[a]
C20:4n6	0.42 ± 0.01	ND
C22:2n6	0.77 ± 0.05[a]	0.14 ± 0.01[a]
Σ PUFA n-6	**16.98 ± 0.14[a]**	14.74 ± 0.58[a]
C18:3n3	2.24 ± 0.24[a]	4.05 ± 0.09[b]
C20:5n3	0.55 ± 0.04[a]	0.48 ± 0.02[a]
C22:6n3	6.50 ± 0.21[a]	5.52 ± 0.32[a]
Σ PUFA n-3	**9.29 ± 0.38[a]**	10.05 ± 0.42[a]
SFA/PUFA	1.67 ± 0.14[a]	1.89 ± 0.18[a]
n-6/n-3	1.83 ± 0.15	1.47 ± 0.17

Data are expressed as mean ± SE ($n = 15$)
Means of each parameter in the same row with similar superscript are not significantly different

Blood biochemical constituents of *C. mrigala* fingerlings

The blood parameters of *C. mrigala* fed experimental feeds are presented in Table 7. The blood hemoglobin concentration of *C. mrigala* was 4.93 and 4.20 g/dl for control and test feed, respectively, and did not show any significant difference between each other. Moreover, the serum glucose concentration of *C. mrigala* fed control feed (56.26 mg/dl) was statistically similar to that of test feed (55.67 mg/dl). Furthermore, the total serum protein, albumin, and globulin also exhibited similarity between the treatments. The serum triglyceride of *C. mrigala* fed control feed was 100.6 mg/dl which was not significantly different with the triglyceride concentration (99.9 mg/dl) of test feed groups. Similarly, cholesterol, HDL, LDL, and VLDL found independent of two types of feeding treatments with no significant difference was noticed.

Discussion

Cirrhinus mrigala is one of the most important aquaculture species in India and cultured along with two other major carps *Catla catla* and *Labeo rohita* in polyculture systems. It is a detritivorous and naturally accustomed to eating planktons or plant ingredients. In general, global average of 5% fishmeal is used for carp species (Tacon & Metian, 2008). The present experiment was designed to check the complete replacement of FM with plant ingredient mixture in the diet of *C. mrigala*. It was evidenced from available literatures that FM replaced diet with plant ingredients influenced growth, feed efficiency, nutrient digestibility, and body composition of fish (Aksnes, Hope, Høstmark, & Albrektsen, 2006; Fontaínhas-Fernandes, Gomes, Reis-Henriques, et al., 1999). In this study, irrespective of the dietary ingredients composition, attributed similar growth and weight gain for *C. mrigala*. This could be due to the same level of dietary energy (DE) and dietary protein (DP) in both the diets. Preceding studies (Dias et al., 2009; Kaushik, & Cove's, D., Dutto, G.,, & Blanc, D., 2004) suggested that the nutrient makeup of the feed is more important than the ingredients in the feed for the growth performance of fish. Adequate balance of indispensable amino acid profile in the feed can be achieved through a sensible blend of different plant protein sources (Dias et al., 2009; Silva, Espe, Conceição, Dias, & Valente, 2009). In the present study, the test feed, containing different plant ingredient mixture, was probably sufficient to meet the nutrient requirement of the fish species. The specific growth rate and protein efficiency ratio of *C. mrigala* fed these two diets in the present experiment did not vary suggesting the protein utilization from these to feed sources were also similar.

Table 7 Blood biochemical constituents of *C. mrigala* fingerlings fed experimental diets

Particulars	Control feed	Test feed
Hemoglobin (g/dl)	4.93 ± 0.15[a]	4.20 ± 0.20[a]
Glucose (g/dl)	56.26 ± 3.46[a]	55.67 ± 2.52[a]
Protein (g/dl)	3.01 ± 0.04[a]	2.80 ± 0.09[a]
Albumin (g/dl)	1.44 ± 0.08[a]	1.36 ± 0.10[a]
Globulin (g/dl)	1.57 ± 0.35[a]	1.44 ± 0.11[a]
Triglyceride (mg/dl)	100.6 ± 0.91[a]	99.9 ± 3.4[a]
Cholesterol (mg/dl)	91.68 ± 2.31[a]	94.14 ± 1.53[a]
HDL(mg/dl)	77.36 ± 2.78[a]	72.74 ± 1.28[a]
LDL (mg/dl)	74.80 ± 4.82[a]	70.04 ± 3.33[a]
VLDL (mg/dl)	20.12 ± 1.08[a]	19.99 ± 0.67[a]

Means of each parameter in the same row with similar superscript are not significantly different

Digestibility is important in determining the nutrients availability to fish for growth. However, there are many post-absorptive factors that influence nutrient utilization and growth. In addition, the nutrient digestibility is very much dependent on the composition of ingredients used in the feed. In this study, the dry matter and protein digestibility of *C. mrigala* fed the test feed was similar in comparison to FM-based control feed. This indicated the ability of *C. mrigala* in utilizing the plant ingredients efficiently. In preceding studies, the digestibility of plant ingredients such as soybean meal, sesame oil cake, linseed oil cake, and mustard oil cake were reported in range of 76.16 to 84.05% in comparison to 80.23% of FM in carp, rohu (Hossain, Nahar, & Kamal, 1997). It is confirmed from the present study that the carp species can efficiently utilize more than 80% nutrient digestibility from plant ingredients present in their diet. The ADC of protein for two feeds was about 90%, which was similar to protein digestibility of carp-fed plant ingredients in other studies (Hasan, Macintosh, & Jauncey, 1997). The ADC of lipid in both the feeds were around 90%, which is similar to the reported values in other fishes such as silver barb (93.6-94.8%) (Mohanta, Mohanty, Jena, & Sahu, 2007), rohu (90.42-94.05%) (Hossain et al., 1997), and mrigal (92.1-98.1%) (Singh, 1991) fed different plant protein-based practical diets. The ADC of energy was around 90% in *C. mrigala* and similar in both the feeds, which were also higher than the reported values in common carp (66.5-81.1%) (Hossain & Jauncy, 1989) fed different plant protein-based diets. The ADCs of protein, lipid, and energy around 90% found in all the diets in our study indicated that the nutrients present in the diets are fairly digestible.

Digestion is the biodynamics of enzymes. The knowledge of digestive enzyme activities helps in selecting the right ingredient for feed formulation for a species. The carbohydrase activity in fish gut is also largely affected by the dietary carbohydrate level and source (Kamalam, Medale, & P, S., 2017). In the present study, both the

feeds contain an equal amount of carbohydrate, which could be the reason for equal amylase activities in *C. mrigala* fed both the feeds. Digestive enzymes are very much dependant on feed composition and age/or stage of development (Chaudhuri, Mukherjee, & Homechaudhuri, 2012; Cousin, Laurencin, & Gabaudan, 1987). The stage of growth of fish used for the present experiment was the same and the equal activities of the digestive enzymes in *C. mrigala* fed both the diets suggested that *C. mrigala* utilized the nutrients irrespective of the source.

In the present study, whole-body proximate compositions of *C. mrigala* did not differ significantly among the dietary treatments. Similar observations were made in the whole-body protein content of Nile tilapia (El-Saidy & Gaber, 2003) and carp (Pongmaneerat, Watanabe, Takeuchi, & Satoh, 1993) when fed plant protein mixture feeds against fish meal containing feeds. The whole body lipid content of *C. mrigala* in the both the treatments were also the same. The presence of fish oil from the fishmeal source was evident with the presence of EPA and DHA in the fatty acid composition of feed (Table 1), however, that did not influence the whole body lipid composition of *C. mrigala*.

The fatty acid profiles of *C. mrigala* were not significantly affected by the dietary treatments except the ALA content. The fish fed with linseed oil supplemented feed deposited a significantly higher amount of ALA in their flesh. This clearly indicated the higher amount of ALA in the test feed resulted in higher flesh ALA content. In this connection, preceding studies (Glencross, 2009; Tocher, 2003; Turchini & Francis, 2009) described that modification of tissue fatty acid in fin fish were possible with the substitution of fish oil with other dietary lipid. However, the possibility of fatty acids undergo changes through metabolic processes, involving utilization for energy production (b-oxidation), bio-conversion (chain elongation and desaturation), and de novo fatty acid production (lipogenesis) cannot be ignored (Tocher, 2003). Linseed oil was used in this study because of the abundance of n-3 PUFA, ALA in linseed oil, precursor of longer, more unsaturated and health-promoting n-3 long-chain-PUFA, EPA, and DHA (Turchini et al., 2009). In the present experiment, the whole body EPA and DHA contents were similar in both the treatments, indicating bioconversion of ALA into LC-PUFAs. There was also no superiority in flesh fatty acid observed in fish fed with control feed which had residual fish oil.

Replacement of FM and FO by plant ingredients did not influence the blood biochemical constituents in *C. mrigala* in the present experiment which agrees to the earlier observations in *C. carpio* (Stepanowska & Sawicka, 2006) fed with fishmeal replaced diet with plant ingredients.

Conclusion

The study indicated that growth, nutrient utilization, body composition, and blood metabolites of Indian major carp, *C. mrigala*, are not influenced by completely replacing fish meal by a mixture of plant protein ingredients in their diets. The nutrient digestibility percentage of both FM-based diet and plant ingredient-based diet was similar which indicated the ability of *C. mrigala* in utilizing the plant ingredients efficiently. The residual fish oil from the FM did not change the fatty acid profile of *C. mrigala* and was similar to the group fed with a linseed oil-based test diet. Supplementation of linseed oil in the feed increased the flesh ALA concentration, without increasing the EPA and DHA levels. This feed is a practical formulation with suitable nutrient digestibility and can be provided to Indian major carps in a polyculture system.

Abbreviations

LC-PUFA: Long-chain polyunsaturated fatty acid; SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; ALA: Alpha-linolenic acid; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid; HDL: High-density lipoprotein; FCR: Feed conversion ratio; SGR: Specific growth rate; PER: Protein efficiency ratio; VSI: Viscero somatic index; HSI: Hepato somatic index; LDL: Low-density lipoprotein; VLDL: Very low-density lipoprotein; BHT: Butylated hydroxytoluene; ADC: Apparent digestibility coefficient; CIFA: Central Institute of Freshwater Aquaculture

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Authors' contributions

Dr. CP conducted the feeding experiment, performed the biochemical analysis, analyzed the results, and drafted the paper. SSG designed the study. SNM provided technical guidance and KCN helped in feeding and biochemical analysis. All authors have read and approved the manuscript.

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Availability of data and materials

Data is available on request.

Ethics approval and consent to participate

The use of *C.mrigala* fingerlings for all experimental purposes was approved (approval number, ICAR-CIFA/Eth/02/2016) by the Ethics Committee of the ICAR (Indian Council of Agricultural Research)-Central Institute of Freshwater Aquaculture (CIFA), Government of India. The fish were used in the present experiment with proper husbandry practices. The dissection procedure was conducted by anesthetizing the control and test group fish with MS222 (Tricaine methanesulphonate, Himedia, India, cat no: RM2178) to minimize pain.

Consent for publication

All authors have given their consent for publication

Competing interests

The authors declare that they have no competing interests.

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References

Acharya, G., & Mohanty, P. K. (2019). Effect of two different habitat on haematological parameters of fishes of Odisha. *Comparative Clinical Pathology*, *28*, 321–330.

Adekoya, A., Porcadilla, M., Varga, D., & Kucska, B. (2018). Replacing fish meal with alternative protein sources in common carp's feed. *Acta agraria aposváriensis*, *22*(2), 18–24. https://doi.org/10.31914/aak.2283.

Aksnes, A., Hope, B., Høstmark, Ø., & Albrektsen, S. (2006). Inclusion level of size fractionated fish hydrolysate in high plant protein diets for Atlantic cod, *Gadus morhua*. *Aquaculture*, 261, 1102-1110. https://doi.org/https://doi.org/10.1016/j.aquaculture.2006.07.038

AOAC (1998). *Official methods of analysis*, (16th ed.,). Arlington: Association of Official Analytical Chemists.

Bergmeyer, H. L. (1974). *Methods of enzymatic analysis*, (vol. 1-4, 2nd ed.,). New York: Academic Press.

Chaudhuri, A., Mukherjee, S., & Homechaudhuri, S. (2012). Diet composition and digestive enzymes activity in carnivorous fishes inhabiting mudflats of Indian Sundarban estuaries. *Turkish Journal of Fisheries and Aquatic Sciences*, *12*, 265–275. https://doi.org/10.4194/1303-2712-v12_2_11.

Christie, W. W. (1982). Lipid analysis. In W. W. Christie (Ed.), *Lipid Analysis*, (vol. 17-23, pp. 51–61). Oxford: Pergamon Press.

Cousin, J. C. B., Laurencin, F. B., & Gabaudan, J. (1987). Ontogeny of enzymatic activities in fed and fasting turbot, *Scophthalmus maximus* L. *Journal of Fish Biology*, *30*, 15–33.

Crampton, V. O., Nanton, D. A., Ruohonen, K., Skjervold, P.-O., & El-Mowafi, A. (2010). Demonstration of salmon farming as a net producer of fish protein and oil. *Aquaculture Nutrition*, *16*(4), 437–446. https://doi.org/10.1111/j.1365-2095.2010.00780.x.

Deng, J., Wang, Y., Chen, L., Mai, K., Wang, Z., & Zhang, X. (2017). Effects of replacing plant proteins with rubber seed meal on growth, nutrient utilization and blood biochemical parameters of tilapia (*Oreochromis niloticus* × *O. aureus*). *Aquaculture Nutrition*, 23, 30-39. https://doi.org/https://doi.org/10.1111/anu.12355

Dias, J., Conceição, L. E. C., Ribeiro, A. R., Borges, P., Valente, L.M.P., Maria, & Dinis, M.T. (2009). Practical diet with low fish-derived protein is able to sustain growth performance in gilthead seabream (*Sparus aurata*) during the grow-out phase. *Aquaculture*, 293, 255-262. https://doi.org/https://doi.org/10.1016/j.aquaculture.2009.04.042, DOI: https://doi.org/10.1007/s00580-018-2844-9

El-Saidy, D. M. S., & Gaber, M. M. A. (2003). Replacement of fish meal with a mixture of different plant protein sources in juvenile Nile tilapia *Oreochromis niloticus* (L.), diets. *Aquaculture Research*, *34*, 1119–1127.

Folch, A. C., Leeds, M., & Sloane-Stanley, G. M. (1957). A simple method for isolation and purification total lipids from animal tissues. *Journal of Biological Chemistry*, *226*, 497–509.

Fontaínhas-Fernandes, A., Gomes, E., Reis-Henriques, M. et al. (1999). Replacement of fish meal by plant proteins in the diet of Nile tilapia: Digestibility and growth Performance. *Aquaculture International,* 7, 57–67. https://doi.org/https://doi.org/10.1023/A:1009296818443

Francis, G., Makkar, H. P. S., & Becker, K. (2001). Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture,* 199, 197-227. https://doi.org/https://doi.org/10.1016/S0044-8486(01)00526-9

Glencross, B. D. (2009). Exploring the nutritional demand for essential fatty acids by aquaculture species. Reviews in Aquaculture, 1, 71-124 https://doi.org/https://doi.org/10.1111/j.1753-5131.2009.01006.x.

Hasan, M. R., Macintosh, D. J., & Jauncey, K. (1997). Evaluation of some plant ingredients as dietary protein sources for common carp (*Cyprinus carpio* L.) fry. *Aquaculture*, 151, 55-70. https://doi.org/https://doi.org/10.1016/S0044-8486(96)01499-8

Hossain, MA, & Jauncy, K. (1989). Studies on the protein, energy and amino acid digestibility of fish meal, mustard oil cake, linseed and sesame meal for common carp (*Cyprinus carpio* L.). *Aquaculture,* 83, 59-72 https://doi.org/https://doi.org/10.1016/0044-8486(89)90060-4.

Hossain, M. A., Nahar, N., & Kamal, M. (1997). Nutrient digestibility coefficient of some plant and animal proteins for rohu (*Labeo rohita*). *Aquaculture*, 151, 37-45 https://doi.org/https://doi.org/10.1016/S0044-8486(96)01481-0.

Hua, K., Cobcroft, J. M., Cole, A., Condon, K., Jerry, D. R., Mangott, A., Praeger, C., Vucko, M. J., Zeng, C., Zenger, K., & Strugnell, J. M. (2019). The future of aquatic protein: Implications for protein sources in aquaculture diets, *One Earth*, 1, (3), 316-329. https://doi.org/https://doi.org/10.1016/j.oneear.2019.10.018

Kamalam, B.S., Medale, F., & P, S. (2017). Utilisation of dietary carbohydrates in farmed fishes: New insights on influencing factors, biological limitations and future strategies. Aquaculture, 467, 3-27. https://doi.org/https://doi.org/10.1016/j.aquaculture.2016.02.007

Kaushik, S. J., & Cove's, D., Dutto, G., & Blanc, D. (2004). Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. *Aquaculture*, *230*, 391–404.

Kriton, G., Dimitra, K., Corraze, G., Jaume, P.-S., Adorjan, A., & Zsuzsanna, J. S. (2018). Impact of diets containing plant raw materials as fish meal and fish oil replacement on rainbow trout (*Oncorhynchus mykiss*), gilthead sea bream (Sparus aurata), and common carp (*Cyprinus carpio*) Freshness. *Journal of food quality*.

Kunitz, M. (1947). Crystalline soybean trypsin inhibitor: II. General properties. *Journal of General Physiology*, *30*, 291–310.

Love, J. (1986). *Clinical chemistry, 32, 1290-1302*.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with Folin Phenol reagent. *Journal of Biological Chemistry.*, *193*, 265–276.

Misra, S., Sahu, N. P., Pal, A. K., Xavier, B., Kumar, S., & Mukherjee, S. C. (2006). Pre- and post-challenge immuno-haematological changes in *Labeo rohita* juveniles fed gelatinised or non-gelatinised carbohydrate with n-3 PUFA. *Fish & Shellfish Immunology*, 21, 346-356. https://doi.org/https://doi.org/10.1016/j.fsi.2005.12.010

Mohanta, K. N., Mohanty, S. N., Jena, J. K., & Sahu, N. P. (2007). Effect of different oil cake sources on growth, nutrient retention and digestibility, muscle nucleic acid content, gut enzyme activities and whole-body composition in silver barb, *Puntius gonionotus* fingerlings. *Aquaculture Research*, *38*, 1702–1713.

Phillips, A. M. (1969). Nutrition, digestion and energy utilization. In W. S. Hoar, & D. J. Randall (Eds.), *Fish Physiology*, (vol. I, pp. 391–432). New York, NY: Academic Press.

Pongmaneerat, J., Watanabe, T., Takeuchi, T., & Satoh, S. (1993). Use of different protein meals as partial or total substitution for fish meal in carp diets. *Bulletin of the Japanese Society for the Science of Fish*, 59, 1249-1257. https://doi.org/https://doi.org/10.2331/suisan.59.1249

Pradhan, C., Giri, S.S., Mohanty, S.N., Narasimmalu, R. (2019). Effect of different feeding levels of plant-ingredient-based feed on fillet fatty acid profile, carcass trait, and sensory characteristics of Indian major carps in earthen pond polyculture. *Journal of the World Aquaculture Society*. 50(2), 374-389. https://doi.org/https://doi.org/10.1111/jwas.12532

Pradhan, C., Giri, S.S. Mohanty, T. K. & Mohanty, S. N. (2020). Influence of a diet containing plant ingredients at different levels on growth performance, carcass biochemical composition, and blood parameters in Indian major carps grown in polyculture earthen ponds. *Tropical Animal Health and Production*. https://doi.org/https://doi.org/10.1007/s11250-019-02184-7.

Pratoomyot, J., Bendiksen, E. Å., Bell, J. G., & Tocher, D.R. (2010). Effects of increasing replacement of dietary fishmeal with plant protein sources on growth performance and body lipid composition of Atlantic salmon (*Salmo salar* L.). Aquaculture, 305, 124-132. https://doi.org/https://doi.org/10.1016/j.aquaculture.2010.04.019

Silva, J. M. G., Espe, M., Conceição, L. E. C., Dias, J., &Valente, L. M. P. (2009). Senegalese sole juveniles (*Solea senegalensis* Kaup, 1858) grow equally well on diets devoid of fish meal provided the dietary amino acids are balanced. *Aquaculture*, 296, 309-317. https://doi.org/https://doi.org/10.1016/j.aquaculture.2009.08.031

Singh, B.N. (1991). Digestibility of lipid in different feeds by mrigal, *Cirrhinus mrigala* (Ham.) and grass carp, *Ctenopharyngodon idella* (Val.), p. 83-86. /n S.S. De Silva (ed.) Fish nutrition research in Asia. Proceedings of the Fourth Asian Fish Nutrition Workshop. Asian Fish. Soc. Spec. Publ. 5, 205 p. Asian Fisheries Society, Manila, Philippines.

Stepanowska, K., & Sawicka, A. (2006). The effect of feeding method on body weight gains, concentrations of selected components in the blood, and peroxidation processes of carp, *Cyprinus carpio*. *Acta Ichthyologica et Piscatoria*, *36*, 25–29. https://doi.org/10.3750/AIP2006.36.1.04.

Tacon, A. G. J., & Metian, M. (2008). Global overview on the use of fish meal and fish oil industrially compounded aquafeeds: Trends and future prospects. *Aquaculture*, *285*, 146–158. https://doi.org/10.1016/j.aquaculture.2008.08.015.

Tacon, A. G. J., & Metian, M. (2015). Feed matters: Satisfying the feed demand of aquaculture. *Reviews in Fisheries Science & Aquaculture*, *23*(1), 1–10. https://doi.org/10.1080/23308249.2014.987209.

Thobaitia, A. A., Ghanima, K. A., Ahmeda, Z., Sulimana, E. M., & Mahboob, S. (2018). Impact of replacing fish meal by a mixture of different plant protein sources on the growth performance in Nile tilapia (*Oreochromis niloticus* L.) diets. *Brazilian Journal of Biology*, *78*(3), 525–534.

Tocher, D. R. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science*. 11, 107-184. https://doi.org/https://doi.org/10.1080/713610925

Turchini, G. M., & Francis, D. S. (2009). Fatty acid metabolism (desaturation, elongation and b-oxidation) in rainbow trout fed fish oil- or linseed oil-based diets. *British Journal of Nutrition*, *102*, 69–81. https://doi.org/10.1017/S0007114508137874.

Turchini, G. M., Torstensen, B. E., & Ng, W. K. (2009). Fish oil replacement in finfish nutrition. *Reviews in Aquaculture*, *1*, 10–57.

Weinrauch AM, Schaefer CM, Goss GG (2019) Activity and post-prandial regulation of digestive enzyme activity along the Pacific hagfish (*Eptatretus stoutii*) alimentary canal. *PLoS ONE*, 14, (4), e0215027. https://doi.org/https://doi.org/10.1371/journal.pone.0215027

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