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Combined plant proteins as fishmeal replacements in pearlspot (*Etroplus suratensis*): Effects on growth performance, digestive enzymes and fatty acid composition

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Abstract

A mix of plant proteins (PP) (soybean meal, groundnut oilcake, wheat gluten meal and corn gluten meal in a 4:2:2:2 ratio) was used to partially replace fishmeal in the diets of pearlspot (*Etroplus suratensis*). Five iso-nitrogenous diets (301.20-306.85 g kg^{-1} crude protein) were prepared by substituting fishmeal at 0% (control, FM-R0), 25% (FM-R25), 50% (FM-R50), 75% (FM-R75) and 100% (FM-R100). The results revealed that the growth performance remained optimal up to 50% fishmeal replacement, beyond which significant declines were observed. Digestive enzyme activities varied significantly ($p<0.05$) with fishmeal substitution levels. Pepsin activity was highest at 50% replacement, while trypsin, chymotrypsin, carboxypeptidase A and B activities were comparable to the control. Pepsin activity was observed only in the stomach, whereas trypsin and chymotrypsin activities were detected in the liver, anterior intestine, and posterior intestine, with the highest activity reported in the posterior intestine Carcass composition in terms of moisture, protein, and ash was significantly influenced ($p<0.05$) by dietary changes, while other parameters remained unaffected. Fatty acids, in particular C20:4, C20:5, and C22:6 were significantly lower ($p<0.05$) in fish fed with higher PP (FM-R75, FM-R100). These results demonstrate that up to 50% of dietary FM can be effectively replaced with a PP blend in pearlspot juveniles without compromising growth, digestive enzyme activity, or carcass quality.

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Introduction

Global aquaculture production in 2022 achieved a record high of 130.9 million t, valued at 312.8 billion USD, solidifying its position as the leading producer of aquatic animals with 94.4 million t, surpassing capture fisheries (FAO, 2024). In growing aquaculture production systems, feed represents a significant input, accounting for 50-60% of operational costs (Biswas *et al*., 2022). However, the feed industry predominantly relies on fishmeal (FM), a major protein source for both omnivorous and carnivorous fish species. Due to its high digestibility, favourable amino acid and fatty acid profiles, FM constitutes approximately 50-70% of the total ingredients used in aquafeeds (Jannathulla *et al*., 2019). However, its limited availability and rising costs necessitate the exploration of alternative protein sources to reduce fishmeal dependency (Xu *et al*., 2024). Plant-based protein (PP) sources have gained significant attention as substitutes for FM due to their affordability, consistent quality and reliable supply (Jannathulla *et al*., 2019). PP sources and byproducts, such as soybean meal and groundnut meal, are recognised as effective sources to replace FM partially in various aquaculture species. Soybean meal has been used to replace 35-75% of FM in the diets of Asian seabass (*Lates calcarifer*) (Yanti *et al*., 2019), milkfish (*Chanos chanos*) (Bharathi *et al*., 2020), stinging catfish (*Heteropneustes fossilis*) (Howlader *et al*., 2023) and tilapia (*Oreochromis niloticus × O. aureus*) (Lin *et al*., 2011). Groundnut meal has replaced 10-15% of FM in the diets of rainbow trout (*Oncorhynchus mykiss*) (Acar *et al*.,

2018) and juvenile hybrid grouper (*Epinephelus fuscoguttatus* ♀×*E. lanceolatus* ♂) (Zhu *et al*., 2022). Similarly, glutens, specifically wheat gluten meal and corn gluten meal, have been identified as promising protein sources, containing 60-80% crude protein and are also used to partially replace FM in aquafeeds (Nandakumar *et al*., 2017). However, the use of these PP sources in aquafeeds has been limited due to deficiencies in essential amino acids, particularly lysine and methionine, as well as the presence of various anti-nutritional factors (Jannathulla *et al*., 2017). To address these challenges, researchers have explored using combinations of PP sources, rather than relying on a single source, with varying degrees of success across different aquatic species. Santigosa *et al*. (2008) reported 75% FM replacement in trout and seabream (*Sparus aurata*) using a PP mixture consisting of corn gluten meal, wheat gluten, extruded peas, and rapeseed meal. Gisbert *et al*. (2016) reported that 75% of FM could be successfully substituted by a PP mixture including corn gluten, wheat gluten, soybean meal and soy protein concentrate in the diets for weaning and on-growing wild fry of grey mullet (*Mugil cephalus*). Abbasi *et al*. (2020) reported 80% FM replacement using a PP mixture (soybean meal and corn gluten) and vegetable oils (canola and soybean oils) in yellowfin seabream (*Acanthopagrus latus*). Madhubabu *et al*. (2021) reported a 50% FM substitution by a blend of PP sources (soybean meal, groundnut oil cake, sunflower oil cake, wheat gluten meal and corn gluten meal) in the diets of Asian seabass.

The pearlspot (*Etroplus suratensis*), a resilient euryhaline species that adapts to both freshwater and brackishwater environments, supports monoculture and polyculture systems (Biswas *et al*., 2013). Valued for its flesh quality and palatability, pearlspot holds significant preference among consumers in South Asia. The efficiency of fish in digesting and absorbing nutrients from feed is determined by the secretion of digestive enzymes within the gastrointestinal system (Jiao *et al*., 2023). Since fish are monogastric, their fatty acid (FA) profiles are affected by the FA composition of the diets they were fed (Fabrikov *et al*., 2021). Research on the combined use of different PP sources as FM substitutes in pearlspot diets is scarce. Hence, this study investigates the impact of incorporating varying levels of multiple PP sources on the growth performance, digestive enzyme activity and fatty acid profile of pearlspot, with a focus on FM replacement.

Materials and methods

Four PP sources, groundnut oil cake, soybean meal, wheat gluten meal, and corn gluten meal, were sourced from local markets in Chennai, India. The ingredients were mixed in a 4:2:2:2 ratio, based on the previous findings of Madhubabu (2019), to substitute fishmeal in the diet of pearlspot. Five iso-nitrogenous (301.20-306.85 g kg^{-1}) and iso-lipidic (70.60-71.11 g kg^{-1}) diets were formulated using locally available ingredients (Table 1), based on our previous study by (Madhubabu, 2019), which identified 300g kg^{-1} crude protein and 70 g kg^{-1} crude lipid as optimal for pearlspot (Lekshmi and Prasad, 2014). The diets used in the present experiment were prepared by replacing FM (w/w) at the rates of 0% (control), 25, 50, 75 and 100% (FM-R0, FM-R25, FM-R50, FM-R75 and FM-R100, respectively). The initial coarse ingredients were finely ground in a micro pulveriser (SR300, Retsch®, Germany) and sieved using a 0.5 mm sieve. After sieving, the ingredients were manually mixed. Subsequently, a vitamin-mineral mix and binder were added as additives, along with fish oil and soy lecithin as sources of lipids. The resulting mixture was homogenised for about 20 min using an electric blender (Philips HL7707/01, 750 watt). To form a dough, the homogenised blend was moistened at a rate of 500 mL kg^{-1} using deionised water. Prior to pelletising, the dough was steamed for 5 min at atmospheric pressure and then pelleted using a handmade tabletop pelletiser (2 mm dia die), fabricated at ICAR-Central Institute of Brackishwater Aquaculture (ICAR-CIBA), Chennai, India. The resulting pellets were further dried at 60 °C in a hot air oven to reduce the moisture content to below 10%. The prepared feeds were stored at -20°C until further use. The proximate composition, essential amino acids and fatty acid composition of the experimental diets are presented in Table 2.

Experimental feeding

Juveniles of pearl spot, obtained from the hatchery of ICAR-CIBA, Chennai, were used in this study. The juveniles were acclimatised for a period of about two weeks to the experimental conditions. During this period, they were fed a formulated feed comprising 300 g kg^{-1} crude protein and 70 g kg^{-1} crude lipid that was developed at ICAR-CIBA. After acclimatisation, 300 healthy and uniformly sized fish (13.23±0.08 g) were stocked randomly into fifteen oval-shaped FRP (fiberglass-reinforced plastic) tanks, each with a capacity of 500 l (1.31m x 0.64m x 0.73 m). All experimental tanks were supplied with sand-filtered seawater (28-29 ppt) and continuous aeration was provided *via* air diffuser stones. The tanks were covered with fibre mats to prevent fish escape and avoid external disturbances. All five treatments contained three replicates and each replicate was stocked with twenty fish. The weight of each individual fish was recorded at the beginning of the experiment. The experiment was carried out for 45 days. Experimental animals were hand-fed twice daily upto satiation at 09:30 and 16:00 hrs. After feeding, any

Table 1. Ingredients of experimental diets containing graded levels of plant protein mix by replacing fishmeal (g kg^{-1} as fed basis)

Ingredients	Experimental diets				
	FM-R0	FM-R25	FM-R50	FM-R75	FM-R100
Fishmeal[1]	150	112.5	75	37.5	-
PPM[2]	0	50	96	142	188
Acetes	60	60	60	60	60
Soybean meal	220	220	220	220	220
Groundnut oil cake	60	60	60	60	60
Rice bran	80	80	80	80	80
Whole wheat	316	301.5	291	280.5	270
Broken rice	50	50	50	50	50
Fish oil[1]	23	24	25	26	27
Soy-lecithin[3]	11	12	13	14	15
Premix[4]	20	20	20	20	20
Binder[5]	10	10	10	10	10

[1]Bismi Fisheries, Mayiladuthurai, Tamil Nadu, India
[2]Plant protein mix composition: Soybean meal, Groundnut oil cake, Wheat gluten meal and Corn gluten meal at the ratio of 4:2:2:2
[3]Real Soy Enterprises, Madhya Pradesh, India
[4]Vitamin mineral mix (mg kg-1 feed): vitamin A - 10 000 IU; Vitamin D - 1500 IU; Vitamin E - 60; Vitamin K - 1.5; Niacin - 200; Riboflavin - 37.5; Calcium pantothenate - 125; Vitamin B12 - 20; Thiamin - 15; Pyridoxine - 15; Folic acid - 5.5; Biotin - 750; Choline chloride - 100; Stable vitamin C - 1000, Cobalt - 0.2; Iodine - 0.6; Copper - 5.0; Iron - 14.0; Manganese - 55.0; Zinc - 24.0); and 1% Dicalcium phosphate
[5]Pegabind: Bentoli Agri Nutrition Asia Pvt Ltd, Singapore

Table 2. Proximate composition, Essential amino acid and Fatty acid compositions of experimental diets containing graded levels of plant protein sources by replacing fishmeal

Parameters	Experimental diets				
	FM-R0	FM-R25	FM-R50	FM-R75	FM-R100
Proximate composition (% as fed basis)					
Moisture	91.8	90.95	91.2	90.6	92.2
Crude protein	306.85	301.2	301.52	301.35	302.63
Ether extract	71.02	71.11	70.78	70.6	70.75
Crude fibre	51.03	51.21	52.2	52.5	53.32
NFE1	373.1	381.42	381.4	393.65	397.6
Total ash	106.2	104.11	102.9	91.3	83.5
Essential amino acids (g per 16 g N)					
Arg	2.14	1.95	1.92	1.85	1.78
His	1.01	0.95	0.94	1.01	0.96
Ile	1.63	1.54	1.59	1.56	1.61
Leu	2.32	2.47	2.63	2.71	2.74
Lys	1.49	1.29	1.04	1.01	0.93
Met	0.69	0.63	0.59	0.61	0.58
Phe	1.49	1.37	1.33	1.44	1.47
Thr	1.64	1.17	1.16	1.12	1.09
Trp	0.43	0.39	0.41	0.43	0.38
Val	1.47	1.32	1.29	1.25	1.18
Saturated fatty acids (SFA) (% of total fatty acids)					
C14:0	5.35	5.09	4.64	4.18	4.1
C15:0	0.37	0.45	0.41	0.37	0.26
C16:0	18.96	20.25	21.55	22.56	23.7
C17:0	0.55	0.52	0.49	0.45	0.46
C18:0	6.33	5.53	5.52	4.81	4.8
C20:0	0.49	0.92	0.63	0.64	0.53
C22:0	0.69	0.87	0.78	0.75	0.65
C24:0	0.58	0.21	0.23	0.2	0.18
Mono unsaturated fatty acids (MUFA) (% of total fatty acids)					
C16:1	5.51	5.46	5.61	5.4	4.87
C17:1	0.33	0.35	0.34	0.32	0.32
C18:1n9	15.18	16.86	17.82	19	20.59
C18:1n7	2.15	2.61	2.41	2.17	2.13
C20:1	1.66	0.74	0.83	0.84	0.92
C22:1	0.2	0.2	0.23	0.19	0.18
C24:1	0.18	0.2	0.16	0.25	0.24
Polyunsaturated fatty acids (PUFA) (% of total fatty acids)					
C18:2n6	14.66	14.66	15.42	16.53	17.69
γC18:3	0.64	0.67	0.57	0.48	0.37
αC18:3n3	2.16	2.08	2.04	2.07	2.17
C20:2n6	0.2	0.21	0.22	0.21	0.24
C20:3n6	0.48	0.46	0.51	0.56	0.64
C20:4n6	2.08	1.72	1.58	1.49	1.49
C20:5n3	7.41	7.15	6.66	5.8	5.65
C22:6n3	4.31	4.18	3.88	3.5	3.53
n3/n6	0.77	0.76	0.69	0.59	0.56

All values are means of three replicates

[1]Nitrogen free extract = 100 – (Moisture + Crude protein + Ether extract + Crude fibre + Total ash)

uneaten feed pellets remaining in the tanks were siphoned out daily using a siphoning tube. The collected pellets were then dried at 60°C in a hot air oven to determine feed intake. The total biomass of each tank was measured at fortnightly intervals to adjust the feed ration based on body weight. During the experimental period, approximately 80% of the culture water was exchanged daily before the first feeding. All water quality indices were maintained at optimal levels, with temperature at 28.57±1.48°C, salinity at 26.24±1.47 ppt, pH at 8.34±0.53, DO at 8.11±0.61 mg l^{-1} and ammonia-N at 0.08±0.01 mg l^{-1}. At the end of the feeding trial, the fishes were anesthetised using 2-phenoxyethanol (0.3 ml l^{-1}) for sampling (Nandakumar *et al*., 2017) and weighed individually. The following growth parameters were then determined: weight gain (WG), average daily growth (ADG), daily growth coefficient (DGC), specific growth rate (SGR), feed conversion ratio (FCR), apparent protein utilisation (APU), protein efficiency ratio (PER) and survival. The calculations for these parameters were as follows:

WG (%) = [Final body weight (g) - Initial body weight (g)] / [Initial body weight (g)] x 100

ADG (mg day^{-1}) = [(Final body weight (g) - Initial body weight (g)] / (Experimental days)

DGC = [Final body $weight^{1/3}$ - Initial body $weight^{1/3}$] / (Experimental days) x 100

SGR = [(ln Final body weight) – (ln Initial body weight)] / (Experimental days) x 100

FCR = Feed intake (g) / Weight gain (g)

APU= Protein gain (g) / Protein intake (g)

PER = Weight gain (g) / Protein intake (g)

Survival (%) = (Final no. of animals) / (Initial no. of animals) x 100

From each replicate, five fish (15 fish per treatment) were dissected to separate the stomach, pyloric caeca, liver and anterior and posterior intestines, to assess the digestive enzyme activities. Additionally, another five fish per replicate, not used for digestive enzyme analysis, were analysed for whole body proximate composition and fatty acid profile.

Biochemical analysis

The proximate composition was assessed using the AOAC (1997) method. For the amino acid analysis, a pre-column derivatisation HPLC gradient system (Shimadzu Corp, LC-30AD) was employed after hydrolysing the samples with 6 N hydrochloric acid in a sealed tube filled with nitrogen for 22 h at 110°C (Finlayson, 1964; Jannathulla *et al*., 2017). Tryptophan, which is sensitive to acid hydrolysis, was quantified using an alkali hydrolysis method followed by spectrophotometric analysis at 500 nm (Sastry and Tammuru, 1985). Fatty acid methyl esters (FAMEs) were prepared according to Metcalfe *et al*. (1966) and quantified using a gas chromatograph (GC-2014 Shimadzu) (Khan *et al*., 2018). The soluble total protein content was estimated based on the method of Lowry *et al*. (1951) using UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan). The activities of various digestive enzymes, including pepsin (Anson, 1938), trypsin (Erlanger *et al*., 1961), chymotrypsin (Hummel, 1959), amylase (Bernfeld, 1955), carboxypeptidase-A (Folk and Schirmer, 1963), carboxypeptidase-B (Folk *et al*., 1960), lipase (Winkler and

Stuckmann, 1979), as well as acid and alkaline phosphatase (Walter and Schutt, 1974) were measured according to the respective protocols with activity expressed as U per mg of protein.

Statistical analyses

The experimental data on growth, whole-body composition and fatty acid profile were statistically analysed by employing one-way ANOVA. Two-way ANOVA was applied with two factors: the level of fishmeal substitution (0, 25, 50, 75 and 100%) and the different parts of the digestive tract such as stomach, pyloric caeca, liver, anterior intestine and posterior intestine, to assess differences in digestive enzyme activity. The Statistical Package for the Social Sciences (SPSS version 25.0) was used to analyse the data and means were compared using Tukey's test at a significance level of $p<0.05$.

Results and discussion

Previous research demonstrated that plant protein blends (*e.g.,* soybean meal, groundnut oilcake, corn gluten meal) effectively replaced up to 50% of FM in diets for Asian seabass without compromising growth performance, nutrient utilisation, or digestive enzyme activity (Madhubabu *et al*., 2021). Similarly, Gisbert *et al*. (2016) reported that a PP mixture successfully replaced 75% of FM in weaning diets for grey mullet fry without any detrimental effects, underscoring the viability of PP blends as sustainable alternatives for brackishwater species. In the present study, pearlspot fed diets with 50% FM (w/w) substitution using a mix of PP (soybean meal, groundnut oilcake, wheat and corn gluten meal) exhibited growth performance (weight gain%, ADG, SGR, DGC) comparable to the FM-based control (FM-R0) diet (Table 3). The WG of fish fed the control diet was 90.49% which was statistically similar to those fed FM-R25 (89.14%) and FM-R50 (90.41%) diets. However, weight gain was significantly ($p<0.05$) reduced to 73.76 and 58.28% in those fed 75 and 100% FM replaced diets, respectively. Notably, despite reductions in lysine and methionine concentrations (from 1.49 to 1.01 g and 0.69 to 0.61 g per 16 g N, respectively) with increasing PP inclusion, growth performance remained unaffected at 50% substitution. This retention of growth highlights the compensatory advantage of combining multiple PP over individual sources to address amino acid deficiencies. However, further increases in FM replacement levels with the PP beyond 50% (*i.e.,* FM-R75 and FM-R100) significantly impaired growth performance ($p<0.05$), likely due to elevated antinutritional factors at higher inclusion levels (Jannathulla *et al*., 2019). The SGR up to 50% replacement groups (1.43) was on par with the control group but declined significantly ($p<0.05$) with higher PP inclusion, consistent with trends observed in grey mullet (Gisbert *et al*., 2016), Asian seabass (Madhubabu *et al*., 2021) and milkfish (Bharathi *et al*., 2020), where SGR decreased at 100% FM replacement. In contrast, Jana *et al*. (2012) reported higher SGR values in 100% fishmeal replacement diets with heat-processed soybean meal compared to the control diet in mullet (*i.e.,* 1.62 in 100% FM replacement *vs* 1.16 in 0% FM replacement) and milkfish (*i.e.,* 1.07 in 100% FM replacement *vs* 0.81 in 0% FM replacement). These discrepancies may stem from species-specific physiological adaptations, differences in fish age/size, or variations in culture conditions and feed processing methods (*e.g.,* thermal treatment mitigating antinutritional factors). The FCR did not differ significantly ($p>0.05$) up to 50% FM substitution, but kept increasing significantly ($p<0.05$) with higher inclusion of PP in diets. These findings align with previous studies (Nandhakumar *et al*., 2017; Madhubabu *et al*., 2021), where protein efficiency ratio (PER) and apparent protein utilisation (APU) remained comparable to the control group at up to 50% FM replacement, indicating efficient nutrient utilisation. However, low PER and APU observed at higher substitution levels may be attributed to elevated ANFs and dietary fibre in plant-based diets, which can impair nutrient absorption and metabolic efficiency (El-Gendy *et al*., 2016). The survival was significantly ($p<0.05$) low in FM-R100 (76.67%) and FM-R75 (85.00%) diet-fed groups and was in the range of 91.67-96.67% in other experimental groups. The reduced survival rates observed at higher FM replacement levels are likely due to decreased palatability and feed intake, resulting from the elevated inclusion of PP in diets (Madhubabu *et al*., 2021).

The capacity of fish to metabolise dietary components is influenced by the physical and chemical nature of the feed, the presence of specific digestive enzymes that facilitate targeted nutrient degradation pathways for optimal utilisation and the inherent ability of the fish to digest particular substrates, which is primarily governed by the availability of these digestive enzymes (Pradhan *et al.,* 2021; Thoufeek *et al*., 2025). Among the digestive enzymes

Table 3. Growth performances of *E. suratensis* fed with experimental diets having graded levels of plant protein mix by replacing fishmeal (n=3)

Parameters	Experimental groups					Pooled SEM±	p value
	FM-R0	FM-R25	FM-R50	FM-R75	FM-R100		
IBW (g)[1]	13.21[a]	13.13[a]	13.17[a]	13.28[a]	13.34[a]	0.02	0.4
FBW (g)[2]	25.16[a]	24.83[a]	25.08[a]	23.07[b]	21.12[c]	0.42	<0.001
Weight gain (%)	90.49[a]	89.14[a]	90.41[a]	73.76[b]	58.28[c]	3.43	<0.001
ADG (mg day^{-1})[3]	265.56[a]	260.07[a]	264.59[a]	217.63[b]	172.81[c]	9.8	<0.001
SGR[4]	1.43[a]	1.42[a]	1.43[a]	1.23[b]	1.02[c]	0.43	<0.001
DGC[5]	1.26[a]	1.24[a]	1.25[a]	1.06[b]	0.87[c]	0.04	<0.001
FCR[6]	1.97[c]	2.00[c]	2.00[c]	2.29[b]	2.58[a]	0.06	<0.001
PER[7]	1.69[a]	1.67[a]	1.67[a]	1.46[b]	1.29[c]	0.04	<0.001
APU[8]	33.70[a]	33.97[a]	33.86[a]	29.66[b]	26.23[c]	0.48	<0.001
Survival (%)	96.67[a]	95.00[a]	91.67[a]	85.00[ab]	76.67[b]	2.24	0.004

Means bearing different superscript letters within in the row differ significantly ($p<0.05$)
[1]Initial body weight; [2]Final body weight; [3]Average daily growth; [4]Specific growth rate; [5]Daily growth coefficient; [6]Feed conversion ratio; [7]Protein efficiency ratio; [8]Apparent protein utilisation

analysed, the activity of pepsin was detected only in the stomach and remained significantly higher ($p<0.05$) in treatments with up to 50% FM replacement (2.39-2.61 U mg protein^{-1}). Beyond 50% FM substitution, pepsin activity declined significantly ($p<0.05$) (Table 4). In yellowfin seabream, Abbasi *et al*. (2020) reported an increase in pepsin activity with up to 60% substitution of FM with PP, followed by a decline at higher substitution levels. This suggests that higher levels of FM substitution with PP may influence pepsin activity, potentially due to the presence of ANFs in PP. The activities of the enzymes trypsin and chymotrypsin were highest in the posterior intestine (3.13 and 1.56 U mg protein^{-1}, respectively) but decreased significantly ($p<0.05$) with FM replacement levels exceeding 25%. These findings contrast with those of Gisbert *et al*. (2016), who reported no significant differences in trypsin activity in grey mullet fry fed diets containing up to 75% FM replacement using PP sources. The reduced trypsin and chymotrypsin activities observed at higher PP inclusion levels in the current study might be attributed to elevated levels of antinutritional factors, particularly protease inhibitors, which are abundant in SBM. Soybeans contain two types of protease inhibitors, including the Kunitz trypsin inhibitor (KTI) and the Bowman-Birk inhibitor (BBI). The KTI molecule binds to either trypsin or chymotrypsin, with a higher affinity for trypsin. In contrast, the BBI possesses two active sites, enabling it to simultaneously inhibit both trypsin and chymotrypsin (Dipietro and Liener, 1989). Additionally, Lin and Luo (2011) reported a decline in intestinal and hepatopancreatic protease activity with higher levels of FM substitution by SBM in the diets of juvenile tilapia. Similar findings were observed in juvenile Chinese sucker (*Myxocyprinus asiaticus*) (Yu *et al*., 2013). Amylase activity was detected in all analysed digestive parts, with the highest activity observed in the posterior intestine (8.356 U mg protein^{-1}). The amylase activity increased significantly ($p<0.05$) with the increasing level of FM substitution, reaching its peak in the FM-R100 group. This finding is consistent with Abbasi *et al.* (2020) who reported a significant ($p<0.05$) rise in amylase activity in yellowfin seabream when FM was replaced with PP in their diets. However, the amylase activity was not significantly affected by FM substitution in grey mullet (Gisbert *et al*., 2016). In addition, Pradhan *et al*. (2021) found no significant differences in amylase activity in mrigal (*Cirrhinus mrigala*) when FM was fully replaced with SBM and mustard oil cake. These studies are contrary to the results of our study. Pradhan *et al*. (2021) attributed the unchanged amylase activity to the equal amounts of nitrogen-free extract (NFE) in the experimental diets. In contrast, in our study, the NFE content increased with higher inclusion of plant proteins, which likely contributed to the increased amylase activity observed. Also, the presence of indigestible carbohydrates in PP, such as non-starch polysaccharides, might elevate the amylase activity when included at higher levels in diets (Abbasi *et al*., 2020). Carboxypeptidase A, carboxypeptidase B and acid phosphatase activities were detected in the stomach, anterior intestine, and posterior intestine. The highest activities of carboxypeptidase A (7.24 U mg protein^{-1}) and carboxypeptidase B (8.13 U mg protein^{-1}) were found in the posterior intestine, while acid phosphatase activity (3.549 U mg protein^{-1}) was highest in the anterior intestine. A decreasing trend in the activities of carboxypeptidase A, carboxypeptidase B and acid phosphatase was observed with increasing levels of FM replacement. Alkaline phosphatase activity was detected only in the anterior and posterior intestines, with higher activity observed in the anterior intestine (7.338 U mg protein^{-1}) compared to the posterior intestine (5.029 U mg protein^{-1}). Fish fed the FM-R0 diet exhibited significantly higher alkaline phosphatase activity compared to the other groups. This aligns with the findings of Estévez *et al.* (2011), who reported a decrease in alkaline phosphatase activity in meagre (*Argyrosomus regius*) with higher levels of FM substitution by PP. Lipase activity was present in all digestive parts, with maximum activity (0.994 U mg protein^{-1}) found in the posterior intestine, while, lowest activity was reported in FM-R75, diet-fed group.

Carcass proximate composition of pearlspot fed experimental diets with varying levels of FM replacement is shown in Table 5.

Table 4. Digestive enzymes activities (U mg protein^{-1}) in various digestive tissues of *E. suratensis* fed with experimental diets having graded levels of plant protein mix by replacing fishmeal (n=3)

Particulars	Digestive enzymes								
	Pepsin	Trypsin	Chymotrypsin	Amylase	Carboxy-peptidase A	Carboxy-peptidase B	Acid phosphatase	Alkaline phosphatase	Lipase
Experimental diets (A)									
FM-R0	2.39^{a}	2.49^{a}	1.25^{a}	4.92^{d}	4.9^{a}	5.58^{a}	2.36^{a}	6.21^{a}	0.56^{ab}
FM-R25	2.52^{a}	2.49^{a}	1.24^{ab}	4.93^{c}	4.89^{ab}	5.58^{ab}	2.35^{ab}	6.19^{b}	0.56^{a}
FM-R50	2.61^{a}	2.47^{b}	1.23^{b}	4.96^{b}	4.88^{bc}	5.57^{bc}	2.34^{b}	6.19^{b}	0.56^{a}
FM-R75	1.74^{b}	2.45^{c}	1.21^{c}	4.97^{a}	4.87^{c}	5.56^{c}	2.33^{c}	6.18^{bc}	0.55^{b}
FM-R100	1.47^{b}	2.44^{d}	1.21^{c}	4.98^{a}	4.87^{c}	5.56^{c}	2.31^{d}	6.16^{c}	0.56^{ab}
Digestive parts (B)									
Stomach	2.14	-	-	2.36^{d}	2.25^{c}	2.34^{c}	1.11^{c}	-	0.34^{d}
Liver	-	1.73^{c}	0.87^{c}	2.76^{c}	-	-	-	-	0.39^{c}
Anterior intestine	-	2.54^{b}	1.26^{b}	6.33^{b}	5.13^{b}	6.24^{b}	3.55^{a}	7.34^{a}	0.5^{b}
Posterior intestine	-	3.13^{a}	1.56^{a}	8.36^{a}	7.24^{a}	8.13^{a}	2.35^{b}	5.03^{b}	0.99^{a}
P value									
A	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.026
B	-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
A × B	-	<0.001	<0.001	<0.001	0.018	0.46	0.211	<0.001	0.387
Pooled SEM (±)	0.031	0.087	0.043	0.325	0.308	0.363	0.15	0.214	0.033

Means bearing different superscript letters in a column within main effects and interactions between the categories differ significantly ($p<0.05$)

Fish fed 50 and 75% fishmeal-replaced diets showed significantly (p<0.05) lower moisture content than other groups. The carcass protein content was significantly higher in FM-R25 and FM-R50 compared to other groups (p<0.05) and significantly lower protein content was found in FM-R100 group. Similar results have been reported when FM was replaced using SBM in stinging catfish (*H. fossilis*) (Howlader *et al*., 2023). Carcass lipid, crude fibre and NFE content were not affected (p>0.05) due to modification, while total ash was gradually (p<0.05) increased with increasing FM replacement.

In the present study, dietary modifications influenced the carcass fatty acid (FA) profile, with C16:0, C16:1, C18:0, C18:1n-9, C18:2n-6, and C22:6 being the most predominant FAs, collectively accounting for over 50% of the total FA composition (Table 6). These findings align with observations reported in other fish species, such as grey mullet (Gisbert *et al*., 2016), Asian seabass (Madhubabu *et al*., 2021), European seabass (Messina *et al*., 2013), Atlantic salmon (Pratoomyot *et al*., 2010) and Senegalese sole (Cabral *et al*., 2011). FAs including C16:1, C20:4, C20:5 and C22:6 showed a significant reduction (p<0.05) as FM substitution levels increased, while C18:1n-9 and C18:2n-6 displayed an inverse pattern. These observations are consistent with findings reported in Asian seabass under similar dietary regimes (Madhubabu *et al*., 2021). As monogastric organisms, fish exhibit tissue FA profiles that closely reflect dietary intake (Fabrikov *et al*., 2021), influencing the direct influence of feed composition on carcass FA dynamics. Fish fed the FM-R100 diet with complete FM replacement exhibited significantly (p<0.05) lower levels of EPA and DHA compared to other dietary treatments, even though fish oil was added in the diet to overcome their deficiency in diets. However, the total polyunsaturated fatty

Table 5. Carcass proximate composition (%) of *E. suratensis* fed with experimental diets having graded levels of plant protein mix by replacing fishmeal (n=3)

Parameters	Experimental groups					Pooled SEM ±	p value
	FM-R0	FM-R25	FM-R50	FM-R75	FM-R100		
Moisture	70.72a	70.61a	70.12b	70.22b	70.69a	0.073	0.001
Crude protein	18.03ab	18.25a	18.22a	18.06ab	17.81b	0.046	0.002
Ether extract	4.68a	4.50a	4.44a	4.38a	4.22a	0.054	0.073
Crude fibre	0.94a	0.98a	0.94a	0.91a	0.91a	0.011	0.388
NFE[1]	0.62a	0.63a	0.90a	0.92a	0.84a	0.059	0.335
Total Ash	5.01b	5.04b	5.39a	5.51a	5.52a	0.062	<0.001

Means bearing different superscript letters within in the row differ significantly (p<0.05)
[1]Nitrogen free extract (Calculated by difference)

Table 6. Fatty acid profile of *E. suratensis* fed with experimental diets having graded levels of plant protein mix by replacing fishmeal (n=3)

Fatty acid	Saturated fatty acids (SFA) (% of total fatty acids)					Pooled SEM ±	p value
C14:0	4.25c	4.79a	4.35bc	4.46b	4.88a	0.067	<0.001
C15:0	0.72a	0.70ab	0.63b	0.67ab	0.72ab	0.011	0.033
C16:0	17.34a	17.30a	16.33a	17.14a	17.54a	0.14	0.25
C17:0	0.59a	0.58a	0.61a	0.57a	0.65a	0.014	0.427
C18:0	6.34a	5.87d	6.02c	5.94cd	6.23b	0.047	<0.001
C20:0	0.80a	0.72c	0.78ab	0.74bc	0.75abc	0.009	0.008
C22:0	0.69ab	0.56c	0.71ab	0.60bc	0.78a	0.022	0.001
Monounsaturated fatty acids (MUFA) (% of total fatty acids)							
C16:1	7.46a	7.69a	6.62b	6.55b	6.43b	0.152	0.001
C17:1	0.32a	0.30a	0.29a	0.29a	0.31a	0.006	0.454
C18:1n9	11.61c	12.11bc	12.37b	12.18bc	13.14a	0.143	<0.001
C18:1n7	3.67a	3.27c	3.18c	3.12c	3.45b	0.055	<0.001
C20:1	0.55a	0.53a	0.57a	0.51a	0.55a	0.007	0.056
C22:1	0.13a	0.18a	0.16a	0.16a	0.17a	0.005	0.094
C24:1	0.19b	0.20ab	0.25a	0.20ab	0.20ab	0.007	0.023
Polyunsaturated fatty acids (PUFA) (% of total fatty acids)							
C18:2n6	15.13c	16.67ab	15.86bc	17.13a	17.60a	0.252	<0.001
C20:2n6	0.14a	0.13a	0.13a	0.13a	0.11a	0.007	0.881
γC18:3	0.79b	0.85ab	0.81ab	0.88a	0.86ab	0.011	0.034
αC18:3n3	1.16b	1.22ab	1.20ab	1.27a	1.23ab	0.011	0.015
C20:3n6	0.44a	0.42a	0.45a	0.45a	0.45a	0.005	0.529
C20:4n6	2.73a	2.67ab	2.64ab	2.49bc	2.35c	0.04	0.002
C20:5n3	2.96a	2.83bc	2.80b	2.81b	2.39c	0.052	<0.001
C22:6n3	7.10a	7.06ab	6.80ab	6.66ab	6.63b	0.06	0.016
n3/n6	0.61a	0.55bc	0.56b	0.53cd	0.50d	0.01	<0.001

Means bearing different superscript letters within the row differ significantly (p<0.05)

acid (PUFA) content remained unaffected, likely due to the elevated levels of C18:2 n-6. This was reflected from the diet FA profile, since the control diet contains high EPA and DHA, whereas the FM-R100 diet has a higher level of C18:2 n-6. These results are consistent with the findings reported in grey mullet (Gisbert *et al*., 2016), Asian seabass (Madhubabu *et al*., 2021), and gilthead seabream (Izquierdo *et al*., 2003). In the n-6 series of PUFAs, C18:2 was the predominant FA, and its concentration increased significantly ($p<0.05$) with higher inclusion of PP. In contrast, within the n-3 PUFA series, C22:6 was the most abundant FA, but its concentration significantly decreased ($p<0.05$) as PP were included. The total n-6 FAs in the fish carcass showed an upward trend with the increasing inclusion of PP, while the total n-3 FAs exhibited a decreasing trend. Consequently, the n-3/n-6 ratio decreased from 0.61 to 0.50.

The present study demonstrates that a combination of plant protein sources namely soybean meal, groundnut oilcake, wheat gluten meal and corn gluten meal, can effectively replace up to 50% of FM in the diet of pearlspot (*E. suratensis*) without compromising growth performance. Evaluation of digestive enzyme profiles offered important insights into how pearlspot adapts to fishmeal replacement, highlighting changes in enzyme activity triggered by dietary alterations. These results underscore the potential of blended PP formulations as sustainable alternatives to FM in aquafeeds. However, to further optimise FM replacement and enhance nutrient utilisation, future research should focus on strategies such as supplementation with crystalline amino acids, phosphorus, phytase, customised digestive enzymes and solid-state fermentation. These approaches could mitigate the limitations of plant-based ingredients, enabling higher substitution levels and supporting the development of cost-effective, eco-friendly feeds for pearlspot.

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