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Impact of Fishmeal Replacement with Poultry By-product Meal and Fish Protein Hydrolysate: Effect on Growth Performance, Nutrient Utilization, Whole-Body Composition and Growth Gene (*IGF-1*) Expression of Striped Murrel (*Channa striata*)

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Key words
Channa striata*, Animal by-products, Poultry by-product meal (PBM), Fish protein hydrolysate (FPH), Growth performance, Growth gene expression *(IGF-1)

ABSTRACT

A 60-day feeding trial was conducted to examine the effects of replacing fish meal (FM) protein with graded levels of poultry by-product meal (PBM), with or without supplementation of fish protein hydrolysate (FPH) on growth, nutrient utilization, whole-body composition, and *IGF-1* mRNA expression of striped murrel (*Channa striata*). Five isonitrogenous (44%, crude protein), isolipidic (11%, crude lipid) and isoenergetic (18 MJ/Kg) diets were formulated to replace 0%, 25% and 50% of FM protein with PBM and PBM supplemented with FPH and the diets were designated as 35 FM (control), 25 PBM, 50 PBM, 25 PBM+FPH and 50 PBM+FPH. Triplicate groups (n=3) of 30 striped murrel juveniles with an average initial weight of 10.02±0.15g were fed with test diets daily thrice until apparent satiation (08:00, 13:00 and 18:00 h). Among the dietary groups, significantly higher ($p < 0.05$) weight gain (41.05±1.38 g), specific growth rate (2.71±0.05 % day^{-1}), and better feed conversion ratio (1.30±0.03) were found in fish fed 50 PBM+FPH diet compared to other diets including control (35 FM). No significant differences ($p > 0.05$) were observed in whole-body composition of striped murrel fed different experimental diets. Moreover, fish fed with 50 PBM+FPH diet resulted significantly ($p < 0.05$) upregulated *IGF-1* (2.04±0.06) mRNA expression. It is concluded that, 50% FM protein can be replaced by PBM with supplementation of FPH in striped murrel diets without any negative impacts on growth, nutrient utilization, whole-body composition and growth gene expression (*IGF-1*) of striped murrel (*Channa striata*).

INTRODUCTION

The striped murrel (*Channa striata*) is an air-breathing freshwater carnivore fish that lives in all types of water bodies, from shallow muddy waters to lakes and rivers (Chen, 1990). As a result, the intensive production of striped murrel has increased worldwide (Hung and Huy, 2007). Striped murrel is a highly carnivore fish species requiring high dietary protein (Samantaray and Mohanty, 1997; Hua *et al*., 2019). In formulated diets, protein is the most expensive nutrient component, and it decides

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almost half of the percentage of feed production cost. Fish meal (FM) is the primary source of protein ingredient in the striped murrel diet because of its palatability and acceptability, providing indispensable amino acids, fatty acids, and unknown growth factors. However, worldwide FM production is continuously decreasing with increasing demand for the expansion of aquaculture and other livestock production (FAO, 2022). Consequently, the striped murrel formulated diet is relatively expensive in the commercial market. Therefore, developing alternative protein ingredients that are cheap, locally available, and less expensive than FM is highly required.

Among animal by-products, poultry by-product meal (PBM) is a highly available, sustainable, and comparatively cheaper ingredient than fish meal. Additionally, it has high nutritional values such as high crude protein (58-65%), protein digestibility values (87-91%) (Bureau *et al*., 1999; Yu, 2004; Irm *et al*., 2020), appropriate levels of essential amino acid and fatty acid profiles, micronutrients such as vitamin and minerals and palatability enhancers (Cruz-Suárez *et al*., 2007). Hence, PBM is proven to be the most efficient protein ingredient as an alternative to FM protein in different carnivore fishes including striped murrel (Abdul-Halim *et al*., 2014), spotted rose snapper (Hernández *et al*., 2014), black sea bream (Irm *et al*., 2020), barramundi (Siddik *et al*., 2019; Chaklader *et al*., 2021) and tilapia (Palupi *et al*., 2020; Sathishkumar *et al*., 2021). Nevertheless, a higher inclusion level of PBM diets hampered the growth performance in many carnivore fishes due to the lack of some essential amino acids, fatty acids, and attractability compared to FM (Nengas *et al*., 1999; Cheng *et al*., 2002; Chaklader *et al*., 2020).

Fish protein hydrolysate (FPH) is a by-product resulting from the solubilization of fish and shellfish proteins into simple fragments such as free amino acids, peptides, and oligopeptides using either chemical (e.g., acid and alkaline) and enzymatic (e.g., protease) treatments (Kristinsson and Rasco, 2000). Due to the positive dietary effects of FPH, it is added as supplement, immune-stimulant, attractant and an alternative ingredient to successfully replace FM partially or completely in different marine and freshwater fishes including Atlantic salmon (Hevrøy *et al*., 2005), red sea bream and olive flounder (Khosravi *et al*., 2015), European seabass (Delcroix *et al*., 2015), juvenile barramundi (Siddik *et al*., 2020), Atlantic cod (Johannsdottir *et al*., 2014) and striped murrel (Siddaiah *et al*., 2022). Moreover, FPH added as a supplement to the low-level FM-based diet has improved the growth and immune status of carnivore fishes like *Channa striata* (Suratip *et al*., 2023) and *Lates calcarifer* (Chaklader *et al*., 2020; Hong *et al*., 2021). Only a limited number of studies have been conducted on PBM as a dietary substitute for FM protein in striped murrel. Moreover, FPH supplemented with PBM in the diets of striped murrel has yet to be evaluated. Therefore, this study has been designed to evaluate the effects of dietary substituting FM protein with PBM and supplementation of FPH on growth performance, nutrient utilization, whole-body composition and growth gene (*IGF-1*) expression of striped murrel (*Channa striata*).

MATERIALS AND METHODS

Experimental fish and rearing conditions

The striped murrel (*Channa striata*) fingerlings (2.43±0.35 g) were obtained from Maria Integrated Fish Farm, Thiruvannamalai district, Tamil Nadu, India. Initially, the striped murrel fingerlings were acclimated to experimental conditions for a month in nursery tanks (4m x 4m x 1.5m). During the acclimatization period, the fish were fed with a commercial diet containing 44% crude protein and 10% crude lipids (Growel Feeds Pvt. Ltd., Andhra Pradesh, India) five times per day till satiation to reduce the cannibalistic activity of striped murrel. After acclimatization, a total number of 450 striped murrel juveniles (initial weight of 10.02±0.15g) were randomly distributed into 15 experimental cages (1m x 1m x 1.5 m) at the stocking density of 30 fish/cage. The experimental cages were installed in a cement tank (4m x 4m x 1.5m). Totally 15 experimental cages were utilized for this study, each treatment performed with three replicates (n=3). The fish were fed to satiety thrice daily at 08.00, 13.00, and 18.00 h for 60 days. During the feeding trial, the water quality parameters such as temperature, dissolved oxygen, pH, ammonia-N, nitrite-N, hardness, and total alkalinity were measured, and it was maintained at the levels of 29.65±0.29°C, 5.53±0.25 mg/L, 8.05±0.25, 0.58±0.28 mg/L, 0.48±0.15 mg/L, 175±15 mg/L and 122±5 mg/L, respectively. All the water quality parameters were analysed using following standard protocols of APHA (2012).

Experimental diet preparation and feeding trial

The PBM and FPH were procured from M/s Pragathi Broilers Pvt Ltd, Chennai, India, and M/s Janatha Fishmeal and Oil Products, Pvt. Ltd. Karnataka, India, respectively. Five experimental diets were formulated and prepared to be nearly isoproteic (44%, crude protein), isolipidic (11%, crude lipid), and isoenergetic (18 MJ/kg, gross energy) containing graded levels (25% and 50%) of PBM and supplemented with FPH. The control diet contained 35% of FM and the first two test diets replaced the FM protein with PBM at levels of 25% and 50% and the other two test diets replaced the FM protein with PBM at levels

of 25% and 50% and each diet were supplemented with 3.5% of FPH. The experimental diets were designated as 35 FM (control), 25 PBM, 50 PBM, 25 PBM + FPH, and 50 PBM + FPH. The ingredient and nutritional composition of experimental diets are shown in Table II. The experimental diets were prepared using a twin-screw extruder machine (Jinan Sunpring Machinary, China) in Aqua Feed Extrusion Mill at the Directorate of Incubation and Vocational Training in Aquaculture (DIVA), TNJFU, Chennai, Tamil Nadu, India. All the dry feed ingredients were finely ground with a pulverizer, seized through 180-micron mesh, then added appropriate level of water and thoroughly mixed with a vertical feed mixer. The mixed ingredients were manually transferred into the hopper of the twin screw extruder and extruded through a 2-mm die. During the extrusion, the temperature, pressure, and moisture of the extruder were maintained at levels of 90-95°C, 2-3 bars, and 15-17%, respectively. Then the extruded pellets were dried using a drier at 60°C for 15 min. After drying, the dried pellets were allowed to be coated with fish oil and palm oil using a Pegasus® vacuum coater (KK Life Sciences, Chennai, India). Finally, the dried pellets were packed in plastic containers and stored at 4°C until used.

Table I. Proximate and amino acid composition (%, dry weight) of feed ingredients for production of experimental diets.

Compositions	Fish meal	PBM	FPH	Soybean meal	Squid meal	Corn gluten	Wheat flour	Broken rice
Proximate composition								
Dry matter	89.4	93.2	94.3	92.7	89.9	92.7	90.1	90.3
Crude protein	65.03	58.5	80.3	50.6	47.54	63.7	12.21	10.06
Crude lipid	8.3	18.0	1.1	1.99	6.54	2.5	2.78	1.82
Crude fibre	< 1.0	1.28	< 1.0	4.77	1.0	< 1.0	1.10	< 1.0
Total ash	12.88	7.3	13.5	6.31	19.39	1.25	0.92	0.58
Gross energy (MJ/Kg)	19.20	22.66	19.43	18.41	16.58	20.27	16.42	16.14
Essential amino acids								
Arginine	3.8	5.5	5.49	5.11	4.23	2.01	0.55	0.64
Histidine	1.7	1.03	1.75	1.03	1.02	1.29	0.2	0.2
Isoleucine	2.8	2.6	3.02	1.63	2.78	2.54	0.31	0.33
Leucine	6.4	4	6.3	2.87	4.36	11.36	0.55	0.63
Lysine	5.1	2.1	7.83	1.07	4.69	0.93	0.25	0.3
Methionine	1.7	1.05	3.22	1.2	1.56	1.43	0.12	0.18
Phenylalanine	2.6	2.56	3.83	1.96	2.31	3.59	0.5	0.37
Threonine	2.7	2.11	4.5	1.5	2.06	2.17	0.25	0.33
Tryptophan	0.9	0.37	1.8	0.6	0.2	0	0.12	0
Valine	2.8	3.14	3.3	2.06	2.63	3.06	0.37	0.54
Non-essential amino acids								
Alanine	4.3	1.8	4.89	2.01	3.26	5.76	0.27	0.45
Aspartic acid	6.2	3.29	7.58	5.4	4.58	3.83	0.4	0.81
Cystine	1.1	1.31	0.85	0.69	0.1	1.05	0.25	0.07
Glutamic acid	8.1	5.16	9.57	8.32	7.61	12.23	2.95	1.25
Glycine	5	4.15	6.94	1.99	2.36	1.66	0.31	0.35
Serine	2.5	4.86	4.29	2.31	1.96	3.29	0.43	0.41
Tyrosine	2.1	4.75	3.76	7.8	1.85	3.42	0.32	0.36
Total sum of amino acids	59.8	49.78	78.92	47.55	47.56	59.62	8.15	7.22

PBM, Poultry by-product meal; FPH, Fish protein hydrolysate.

Table II. Ingredient and nutrient composition (%, dry weight) of experimental diets.

Ingredients	Experimental diets				
	35 FM	25 PBM	50 PBM	25 PBM+ 3.5 FPH	50 PBM+ 3.5 FPH
Fish meal[1]	35	26.25	17.5	25.03	16.28
Poultry by-product meal[2]	0	9.72	19.44	9.72	19.44
Fish protein hydrolysate[3]	0	0	0	1	1
Soybean meal[4]	17	17	17	17	17
Squid meal[5]	9	9	9	9	9
Corn gluten[6]	10	10	10	10	10
Wheat flour[7]	11	11	11	11	11
Broken rice[8]	6.2	6.43	6.46	6.65	6.68
Fish oil[9]	3	3	3	3	3
Palm oil[10]	2.2	1.2	0.2	1.2	0.2
Soy lecithin[11]	2	2	2	2	2
Di-calcium phosphate[12]	1	1	1	1	1
Vitamin mix[13]	1	1	1	1	1
Mineral mix[14]	1	1	1	1	1
Vitamin – C[15]	0.2	0.2	0.2	0.2	0.2
DL-Methionine[16]	0.4	0.2	0.2	0.2	0.2
Pega bind[17]	1	1	1	1	1
Nutrient composition (%)					
Dry matter	90.18	89.88	90.36	90.28	90.13
Crude protein	43.76	43.88	43.92	43.98	44.08
Crude lipid	10.91	10.72	10.93	10.85	11.06
Crude fibre	1.16	1.25	1.35	1.09	1.1
Total ash	10.83	10.88	11.55	11.43	11.01
Calcium	1.88	1.69	1.50	1.66	1.47
Phosphorus	1.37	1.24	1.11	1.23	1.10
Gross energy (MJ/kg)	18.33	18.04	17.9	18.07	18.14

FM, fish meal; PBM, poultry by-product meal; FPH, fish protein hydrolysate. [1]Bismi Fisheries Pvt. Ltd., Mayiladuthurai, Tamil Nadu, India. [2]Pragathi broilers Pvt Ltd., Chennai, India. [3]Janatha Fishmeal and Oil Products, Pvt. Ltd. Karnataka, India. [4]Mahindra feeds Pvt. Ltd., Namakkal, Tamil Nadu, India. [5]Mahindra feeds Pvt. Ltd., Namakkal, Tamil Nadu, India. [6]SPAC Starch Products (India) Pvt Ltd., Erode, Tamil Nadu, India. [7]Mahindra feeds Pvt. Ltd., Namakkal, Tamil Nadu, India. [8]Mahindra feeds Pvt. Ltd., Namakkal, Tamil Nadu, India. [9]Bismi Fisheries Pvt. Ltd., Mayiladuthurai, Tamil Nadu, India. [10]Mahindra feeds Pvt. Ltd., Namakkal, Tamil Nadu, India. [11]Otto chemie Pvt. Ltd., Mumbai, India. [12, 15]Jain industrial chemicals, Chennai, India. [13]Anicare Pvt. Ltd., Chennai, Tamil Nadu, India. Composition of vitamin premix (quantity/Kg): Vit. A-10,000,000 IU, Vit. B1-5000 mg, Vit. B2-5000 mg, Vit. B3-6000 mg, Vit. B5-6000 mg, Vit. B6-6000 mg, Vit. C-60,000 mg, Vit. D3-2,000,000 IU, Vit. E-10,000 IU, Vit. H-200 mg. [14]Anicare Pvt. Ltd., Chennai, Tamil Nadu, India. Composition of mineral premix (quantity/kg): Magnesium-2800 mg, Iodine-7.4 mg, Iron-7400 mg, Copper-1200 mg, Manganese-11,600 mg, Zinc-9800 mg, Chlorides cobalt-4 mg, Potassium-100 mg, Selenium-4 mg, Calcium carbonate-27.25%, Phosphorous-7.45 mg, Sulphur-0.7 mg, Sodium-6 mg, Calpan-200 mg, Aluminium-1500 mg and Choline chloride-10,000 mg. [16]Evonik AG (DL-methionine: MetAMINO® – 99%). [17]PEGABIND®, Bentoli Agrinutrition India Pvt. Ltd., Chennai, India.

Growth performance and nutrient utilization analysis

At end of the feeding trial, all fish in each triplicate were starved for 24 h and then anesthetized using tricaine methanesulfonate (MS-222, Sigma-Aldrich Inc.). All fish from each treatment were counted and weighed to estimate the growth parameters such as weight gain (WG), feed intake (FI), percentage weight gain (PWG), average daily growth (ADG), feed conversion ratio (FCR), survival rate (SR), protein efficiency ratio (PER) and specific growth rate (SGR).

The growth performance and nutrient utilization of fish in this feeding trial were calculated as follows:

WG (g) = Final body weight (g) - Initial body weight (g)

FI (g fish^{-1}) = (Dry feed intake (g)/Final body weight (g)/Days of fed)

ADG (g) = (Mean final weight (g) - Mean initial weight (g))/Days of culture

SR (%) = (Number of fish harvested/Number of fish stocked) × 100

SGR (% day^{-1}) = [(LN (final weight)) - (LN (initial weight)/Number of days)] × 100

Hepatosomatic index (HSI, %) = [Weight of liver (g) / Total weight of fish (g)] × 100

Viscerosomatic index (VSI, %) = [Weight of viscera (g) / Total weight of fish (g)] × 100

PER = Wet weight gain of fish (g)/Protein intake (g)

Lipid efficiency ratio (LER) = Wet weight gain of fish (g)/Lipid intake (g)

Protein retention (PR, %) = 100 × [(Final body weight × Final body protein) - (Initial body weight × Initial body protein)] / Protein consumed

Lipid retention (LR, %) = 100 × [(Final body weight × Final body lipid) - (Initial body weight × Initial body lipid)] / Lipid consumed

Calcium retention (CR, %) = 100 × [(Final body weight × Final body calcium) - (Initial body weight x Initial body calcium)] / Calcium consumed

Phosphorus retention (PR, %) = 100 × [(Final body weight × Final body Phosphorus) - (Initial body weight × Initial body Phosphorus)]/ Phosphorus consumed

Proximate composition and amino acid analysis

Before feeding trial, 15 fish were ice killed, and whole-body samples were taken for estimation of whole-body proximate composition. At the end of the feeding trial, 3 fish from each replicate were ice killed and whole-body samples were taken for whole-body proximate composition. The proximate composition of experimental diets and whole-body samples was estimated following the procedure of AOAC (2010). The moisture level of experimental diets and whole-body were analyzed using

a hot air oven at 105°C for 5-h, the kjeldhal method (Kelplus-Distyl Em Ba, Pelican equipments, Chennai, Tamil Nadu, India), and soxhlet method (Socsplus - SCS 04 AS DLSTS, Pelican equipments, Chennai, Tamil Nadu, India) were used to estimate the crude protein and crude lipid content of experimental diets and whole-body samples. The crude fiber content of experimental diets were determined using fiber cap method (FIBRAPLUS FES 04, Pelican equipments, Chennai, Tamil Nadu, India). The total ash content of experimental diets and whole-body samples were assessed using a muffle furnace at 550°C for 6-h. The gross energy content of experimental diets and fish samples was analysed using a bomb calorimeter (IKA-C 6000, IKA® India Private Limited, Bengaluru, India). The amino acid composition of experimental diets and ingredients were determined by ultra-pressure liquid chromatography (UPLC; Model-Waters ACQUITY-UPLC, Waters), following the method of Ishida *et al*. (1981). In brief, 50 mg of dried samples were transferred to an ampule (Borosil glass) sealed under a stream of nitrogen gas, and the samples were hydrolyzed using 6 N HCl for 24 h at 110°C for neutralization and then filtered with 0.2 µm PTFE filter. The hydrolysis samples were derivatized using AccQ-Tag Ultra Derivatization Kit and separated by Waters ACQUITY UPLC equipped with 2.1 × 100 mm and pore size of 1.7 µm column (AccQ-Tag Ultra C18) following stepwise gradient elution. Amino acid standard H (Product no: WAT088122) was used, and the amino acids were quantified based on the absorbance values at 260 nm measured by a tunable UV detector and analyzed using Empower 2 Software. Amino acid standards were also run simultaneously for calibration. Tryptophan was estimated after alkaline digestion of the sample with lithium hydroxide. he proximate and amino acid composition of feed ingredients and amino acid composition of experimental diets are shown in Tables I and III, respectively.

Quantitative real-time PCR (qRT-PCR) analysis

The total RNA (*IGF-1* gene) was extracted from liver samples of experimental fish using PureZOL™ RNA Isolation Reagent (Bio-Rad) according to the manufacturer's protocol. According to the manufacturer's iScript cDNA Synthesis Kit (Bio-Rad) protocol, the first-strand cDNA was synthesized from 2 µg of total RNA. In total, 20 µl of the quantitative real-time polymerase chain reaction (qRT-PCR) contains 10 µM of each primer (forward and reverse), 20 ng of cDNA template, and 1× SYBR green PCR master mix kit. The qRT-PCR analysis was performed in C1000 Touch thermal cycler-CFX96 Real-time PCR (Bio-Rad). The thermal cycling conditions were initial denaturation at 95 ℃ for 10 min, followed by 40 cycles of 15 s denaturation at 95 ℃, annealing at 60-62 ℃ (depending on the target genes) for 30 s, extension at 72 ℃ for 30 s ended with dissolution curve. The expression levels of *IGF-1* were measured using the formula R = 2−ΔΔCt (Livak and Schmittgen, 2001). The relative expression of the *IGF-1* gene was compared using the housekeeping gene *β-Actin*. The primer sequences (Table IV) and protocols of relative gene expression analysis were acquired by Siddaiah *et al*. (2022).

Table III. Amino acid composition (%, dry weight) of experimental diets.

Amino acids	Experimental diets				
	35 FM	25 PBM	50 PBM	25 PBM+ FPH	50 PBM+ FPH
Essential amino acids					
Arginine	2.88	3.08	3.29	3.20	3.40
Histidine	1.03	0.98	0.93	0.99	0.94
Isoleucine	1.82	1.82	1.83	1.83	1.84
Leucine	4.36	4.19	4.02	3.96	3.78
Lysine	2.53	2.29	2.04	2.53	2.28
Methionine	1.50	1.26	1.21	1.31	1.27
Phenylalanine	1.89	1.91	1.93	1.90	1.92
Threonine	1.65	1.62	1.59	1.69	1.66
Tryptophan	0.43	0.39	0.34	0.45	0.41
Valine	1.95	2.01	2.07	2.00	2.06
Non-essential amino acids					
Alanine	2.77	2.57	2.37	2.52	2.32
Aspartic acid	3.98	3.76	3.53	3.87	3.65
Cystine	0.64	0.67	0.70	0.66	0.69
Glutamic acid	6.56	6.35	6.15	6.21	6.00
Glycine	2.52	2.49	2.46	2.67	2.63
Serine	1.85	2.10	2.35	2.12	2.37
Tyrosine	2.63	2.91	3.18	2.90	3.18
Total sum of amino acids	40.99	40.4	39.99	40.81	40.4

The amino acid composition of experimental diet values are expressed as means of three replicates per treatment (n=3).

Statistical analysis

All data obtained from this study were statistically analyzed using one-way ANOVA following the Duncan multiple range test (Duncan, 1955) using statistical software SPSS 24.0 (SPSS Inc., USA) for Windows to determine the significant difference ($p < 0.05$) among the treatments. All data are expressed as mean values ± standard deviation (SD) (n=3).

Table IV. Primer sequences used for qRT-PCR analysis of striped murrel.

Gene name	Gen Bank number	Primer sequence (5′–3′)
Hepatic insulin like growth factor-1 (*IGF-1*)	*JK 546357.1*	F TCTGTGATGTTGACGAGTGGT R AGCCTGAAATGTTGGGAGTG
β-Actin	*KC 967219*	F GCCTTCCTTCCTTGGTATGG R GTGTTGGCGTACAG GTCCTT

RESULTS

Growth performance and survival rate of striped murrel

The growth performance and survival rate of striped murrel fed different levels of PBM and supplementation of FPH diets are shown in Table V. Significant differences ($p < 0.05$) were observed in growth performances such as FW, WG, FI, ADG and SGR of striped murrel fed different levels of PBM and supplementation of FPH diets. However, no significant differences ($p > 0.05$) were observed in SR, HSI, and VSI and the survival rate was obtained in ranged from 95-98%. Among the dietary groups, significantly higher ($p < 0.05$) growth performance was observed in fish fed 50 PBM+FPH diet compared to other dietary groups including the control diet (35 FM).

Feed conversion, nutrient utilization, and retention of striped murrel

The feed conversion, nutrient utilization, and retention of striped murrel fed different levels of PBM and supplementation of FPH diets are shown in Table VI. Significant differences ($p < 0.05$) were observed in FCR, PER, LER and PR of striped murrel. The best FCR (1.32±0.05 and 1.30±0.03) was observed in fish fed 25 PBM + FPH and 50 PBM + FPH diets than other dietary groups. Significantly higher ($p<0.05$) PER (1.73±0.07 and 1.75±0.04),

Table V. Growth performance and survival rate of striped murrel fed PBM with and without supplementation of FPH diets.

	Experimental diets					p value
	35 FM	25 PBM	50 PBM	25 PBM + FPH	50 PBM + FPH	
Initial weight (g)	10.03±0.16	9.89±0.28	10.19±0.12	9.96±0.08	10.01±0.22	0.430
Final weight (g)	$46.03±1.11^{bc}$	$45.54±1.29^{c}$	$43.91±1.46^{c}$	$48.12±1.05^{b}$	$51.06±1.42^{a}$	<0.001
Weight gain (g)	$36.00±1.26^{bc}$	$35.65±1.56^{bc}$	$33.73±1.45^{c}$	$38.17±1.04^{b}$	$41.05±1.38^{a}$	0.001
Feed intake (g fish^{-1})	$52.16±1.54^{ab}$	$51.27±1.56^{abc}$	$49.35±1.33^{c}$	$52.36±2.98^{bc}$	$53.40±0.65^{a}$	0.026
Average daily growth (g)	$0.60±0.02^{bc}$	$0.59±0.02^{bc}$	$0.56±0.02^{c}$	$0.63±0.02^{b}$	$0.68±0.02^{a}$	0.001
Survival rate (%)	98.33±2.88	96.67±5.77	95.00±5.00	98.33±2.88	98.33±2.88	0.804
Specific growth rate(% day^{-1})	$2.54±0.06^{bc}$	$2.54±0.09^{bc}$	$2.43±0.05^{c}$	$2.62±0.03^{ab}$	$2.71±0.05^{a}$	0.003
Heptosomotic index (%)	1.75±0.03	1.72±0.05	1.71±0.04	1.69±0.01	1.69±0.06	0.529
Viscerosomatic index (%)	6.79±0.29	6.76±0.34	7.05±0.57	6.73±0.12	6.60±0.36	0.677

The growth performance and survival rate values were represented as mean ± SD of three replicates per treatment (n=3) and the values with different superscripts indicate significant differences as determined by Duncan's test ($p < 0.05$).

Table VI. Feed conversion, nutrient utilization and retention of striped murrel fed PBM with and without supplementation of FPH diets.

	Experimental diets					p - value
	35 FM	25 PBM	50 PBM	25 PBM + FPH	50 PBM + FPH	
Feed conversion ratio	$1.45±0.06^{a}$	$1.44±0.07^{a}$	$1.46±0.07^{a}$	$1.32±0.05^{b}$	$1.30±0.03^{b}$	0.013
Protein efficiency ratio	$1.57±0.06^{b}$	$1.58±0.07^{b}$	$1.55±0.08^{b}$	$1.73±0.07^{a}$	$1.75±0.04^{a}$	0.010
Lipid efficiency ratio	$6.28±0.26^{b}$	$6.32±0.29^{b}$	$6.21±0.31^{b}$	$6.90±0.27^{a}$	$6.99±0.15^{a}$	0.010
Protein retention (%)	$27.12±1.21^{c}$	$27.85±2.12^{bc}$	$27.49±0.91^{c}$	$30.28±1.31^{ab}$	$30.91±0.96^{a}$	0.021
Lipid retention (%)	35.32±3.79	35.83±1.50	35.05±0.62	39.48±3.25	39.85±3.30	0.146

Feed conversion, Nutrient utilization and retention values were represented as mean ± SD of three replicates per treatment (n=3) and the values with different superscripts indicate significant differences as determined by Duncan's test ($p < 0.05$).

Table VII. Whole-body proximate composition (%, wet weight) of striped murrel fed PBM with and without supplementation of FPH diets.

	Experimental diets						p - value
	Initial	35 FM	25 PBM	50 PBM	25 PBM + FPH	50 PBM + FPH	
Moisture	72.55	72.11±0.20	71.67±0.62	71.57±0.47	71.83±0.23	71.56±0.42	0.493
Crude protein	16.97	17.21±0.05	17.47±0.43	17.53±0.23	17.42±0.12	17.55±0.29	0.541
Crude lipid	4.61	5.40±0.30	5.44±0.16	5.41±0.26	5.49±0.27	5.49±0.29	0.988
Total ash	4.43	3.65±0.15	3.77±0.18	3.87±0.21	3.72±0.25	3.83±0.19	0.679
Calcium	0.86	1.07±0.02	1.05±0.03	1.04±0.02	1.08±0.03	1.09±0.02	0.257
Phosphorus	0.52	0.60±0.01	0.60±0.02	0.60±0.01	0.61±0.00	0.61±0.01	0.618

Whole-body proximate composition values were represented as mean ± SD of three replicates per treatments (n=3) and the values with different superscripts indicate significant differences as determined by Duncan's test ($p < 0.05$).

LER (6.90±0.27 and 6.99±0.15) and PR (30.28±1.31 and 30.91±0.96) were observed in fish fed 25 PBM+FPH and 50 PBM+FPH diets. However, no significant difference ($p > 0.05$) was found in LR of fish fed different levels of PBM and supplementation of FPH diets.

Whole-body proximate composition of striped murrel

The whole-body proximate composition of striped murrel fed different levels of PBM and supplementation of FPH diets are shown in Table VII. No significant differences ($p > 0.05$) were observed in whole-body proximate composition such as moisture, crude protein, crude lipid, total ash, calcium, and phosphorus content of striped murrel.

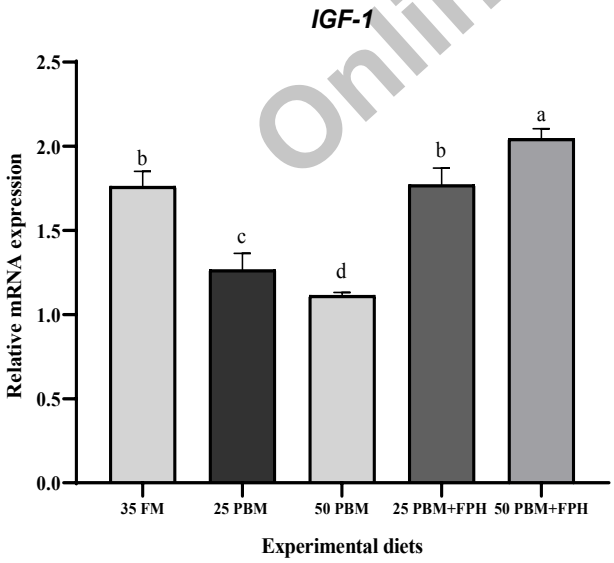

Fig. 1. Relative mRNA expression of *IGF-1* in liver of striped murrel. Bars with different superscripts are significantly (p < 0.05) different.

Relative mRNA expression of IGF-1 *of striped murrel*

The relative mRNA expression of *IGF-1* of striped murrel fed different levels of PBM and supplementation of FPH diets are shown in Figure 1. Significantly ($p < 0.05$) upregulated relative mRNA expression of *IGF-1* (2.04±0.06) levels were observed in fish fed 50 PBM+FPH diets than other diets.

DISCUSSION

The results of the present study showed that dietary FM protein can be replaced with up to 50% of PBM without any adverse effects on the growth performance, feed utilization, and survival rate of striped murrel. Similar to our results, up to 40% PBM is substituted for FM without affecting the growth and feed utilization in the diet of striped murrel (*Channa striata*) fingerlings (Abdul-Halim *et al*., 2014). Hence, the findings of the present study agreed with previous studies with gilthead seabream (*Sparus aurata*) and olive flounder (*Paralichthys olivaceus*), in which growth performance and feed utilization were not significantly affected when FM replaced up to 50% of PBM (Karapanagiotidis *et al*., 2019; Ha *et al*., 2021). Up to 60-80% of FM has been replaced by PBM without any negative effects on growth performance and feed intake in the diets of cobia (Zhou *et al*., 2011), black seabream (Irm *et al*., 2020), barramundi (Siddik *et al*., 2019) and gilthead seabream (Fontinha *et al*., 2021). Additionally, complete replacement of FM with PBM was achieved in diets of humpback grouper (*Cromileptes altivelis*) (Shapawi *et al*., 2007), cobia (*Rachycentron canadum*) (Saadiah *et al*., 2011) and spotted rose snapper (*Lutjanus guttatus*) (Hernández *et al*., 2014) without affect the growth and feed utilization efficiency. Surprisingly, the diets of turbot (*Psetta maeotica*) and large yellow croaker (*Larmichthys crocea*) can be tolerated only a quarter of the amount of PBM instead of FM (Yigit *et al*., 2006; Wang *et al*., 2023).

These different findings from the previous studies could be attributed to various factors such as the source, processed conditions and nutrient composition of PBM, diet preparation methods, protein level of diets, experimental conditions, size and developmental stages of fish, health conditions, and ingredient digestibility capacity of animals (Cheng *et al*., 2002; Krogdahl and Bakke-McKellep, 2005; Dawson *et al*., 2018). In this study, dietary supplementation of FPH in PBM-incorporated diets could enhance the overall growth performance, feed utilization and health conditions of striped murrel compared to other diets. Similarly, Siddik *et al*. (2020), Chaklader *et al*. (2021), and Pham *et al*. (2021) reported that dietary supplementation of tuna hydrolysate and FPH in PBM-incorporated diets could improve the overall growth performance, feed utilization efficiency and health status of Asian seabass (*Lates calcarifer*) and pompano (*Trachinotus blochii*) by eradicating the problems associated with the incorporation of PBM substituted with FM such as lack of digestibility, palatability and certain essential amino acids. Also, it has been experimentally demonstrated in the diets of *Channa striata*, supplementation of 2.5% and 10% FPH in low fish meal diet without affecting the feed intake and growth performance (Siddiah *et al*., 2022; Suratip *et al*., 2023). Hong *et al*. (2021) have demonstrated that the growth performance and feed utilization efficiency were not affected by the replacement of FM with up to 80% of the mixture of PBM and fermented soybean meal supplemented with FPH in the diets of barramundi (*Lates calcarifer*). The fish morphological indices such as HSI and VSI reflect the nutritional status and physiological conditions of reared animals. The values of HSI and VSI in our results were not affected by the partial replacement of FM with PBM, and these findings were confirmed with earlier studies in different carnivore fishes like snakehead (*Channa striata*) (Abdul-Halim *et al*., 2014), great sturgeon (*Huso huso*) (Hassani *et al*., 2021), black seabream (*Acanthoparus schegeli*) (Irm *et al*., 2020), gilthead seabream (*Sparus aurata*) (Sabbagh *et al*., 2019) and barramundi (*Lates calcarifer*) (Siddik *et al*., 2019) when the fishes were fed with different dietary levels of PBM instead of FM.

No significant variations were obtained in the whole-body carcass compositions, such as crude protein, crude lipid, and total ash content of striped murrel when FM protein was replaced with up to 50% of PBM in this study. This is because of dietary groups' isonitrogenous, isolipidic and isocaloric values, and it has been confirmed that whole-body nutrient retention depends on the nutrient composition of dietary groups. Similar to our findings, up to 40% of FM replacement with PBM did not affect the whole-body composition of *Channa striata* fingerlings (Abdul-Halim *et al*., 2014). Previous studies by Karapanagiotidis *et al*. (2019), Irm *et al*. (2020) and Ha *et al*. (2020) showed that the dietary inclusion of PBM does not influence the whole-body composition of various fish species, like gilthead seabream (*Sparus aurata*), black seabream (*Acanthoparus schegeli*) and olive flounder (*Paralichthys olivaceus*). However, including FPH instead of FM did not influence the whole-body composition except for the crude lipid content of *Channa striata* (Siddiah *et al*., 2022). Similarly, up to 60% of PBM-incorporated diets significantly did not impact the whole-body composition, but the crude protein levels were decreased compared to the reference diet of cobia (Zhou *et al*., 2011).

The gene expression analysis is a supporting tool for evaluating the effects of feed ingredients on animal growth performance. *IGF-1* is a growth hormone that is produced in the liver. It is involved and regulated in various physiological functions like cell proliferation, differentiation and division, subsequently increasing the growth and reproduction of mammals, birds and fish (Clemmons and Underwood, 1991; Jones and Clemmons, 1995). The upregulated *IGF-1* mRNA expression levels are positively correlated with the muscle growth development of fish. Among the dietary treatments of this study, *Channa striata* fed with 50% of PBM supplemented with FPH (50 PBM+FPH) diet shows an upregulated expression of *IGF-1* activity compared to other diets. The positive growth performance results obtained might be due to the presence of low molecular weight peptides and free amino acids in the FPH supplemented diet compared to other diets. In line with our present findings, Irm *et al*. (2020) demonstrated higher IGF-1 mRNA expression in fish fed with 30% of PBM-incorporated diets. Furthermore, our results were consistent with the study earlier in Siddaiah *et al*. (2022), which suggested that dietary inclusion of 10% FPH upregulated the *IGF-1* gene in *Channa striata*.

CONCLUSION

In conclusion, the present study revealed that poultry by-product meal could replace up to 50% of fish meal without compromising the growth performance and nutrient utilization of *Channa striata* juveniles. Further studies are required for achieving more than 50% fish meal replacement with PBM supplement with FPH.

DECLARATIONS

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Data availability statement

The data that support the findings of this study are available within the article.

Statement of conflict of interest

The authors have declared no conflict of interest.

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