Pakistan J. Zool., pp 1-11, 2024. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20240516094829

Physio-Metabolic Responses of Striped Murrel (*Channa striata*) Fed on Poultry By- product Meal and Fish Protein Hydrolysate

Govindharaj Sathishkumar[1,2]*, Nathan Felix[3], Amit Ranjan[1], Arumugam Uma[4] and Elangovan Prabu[2]

[1]*Institute of Fisheries Post Graduate Studies, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Chennai - 603 103, Tamil Nadu, India.*
[2]*Directorate of Incubation and Vocational Training in Aquaculture, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, ECR-Muttukadu– 603 112, Tamil Nadu, India.*
[3]*Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam– 611 002, Tamil Nadu, India.*
[4]*Dr. M. G. R. Fisheries College and Research Institute, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Ponneri– 601 204, Tamil Nadu, India*

Article Information
Received 16 May 2024
Revised 03 July 2024
Accepted 09 July 2024
Available online 25 November 2024 (early access)

Authors' Contribution
GS: Conducted the experiment, data interpretation and analysis, drafting - original manuscript; NF: Conceptualization, supervision, manuscript correction; AR: Writing - review and editing, manuscript correction; AU: Assessment of gene expression analysis and data interpretation; EP: Writing - review and editing.

Key words
Animal by-product, *Channa striata*, Fishmeal, Fish protein hydrolysate (FPH), Innate immunity, Poultry by-product meal (PBM)

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 digestive enzyme activities, intestinal morphology, antioxidant and metabolic functions of liver and haemato-biochemical responses of striped murrel (*Channa striata*). Five isonitrogenous (44%, crude protein), isolipidic (11%, crude lipid) and isoenergetic (18 MJ/Kg) diets were formulated such that the FM protein (35%) in the control (35 FM) diet was replaced with only PBM at 25% (25 PBM) and 50% (50 PBM), and PBM supplemented with FPH at 25% (25 PBM+FPH) and 50% (50 PBM+FPH). Triplicate groups (n=3) of 30 striped murrel juveniles (10.02±0.15g) were fed with experimental diets daily thrice (08:00, 13:00 and 18:00 h) until apparent satiation. Among the dietary groups, significantly ($p < 0.05$) higher protease content was observed in fish fed control (35 FM) (6.79±0.39 U mg^{-1} protein), 25 PBM+FPH (7.03±0.38 U mg^{-1} protein) and 50 PBM+FPH (6.96±0.31 U mg^{-1} protein) diets than in other diets. However, amylase and lipase activities were not affected either with the different inclusion levels of PBM and the supplementation of FPH. No significant ($p > 0.05$) differences were observed in SOD, GPx and MDH activities of striped murrel fed experimental diets. FPH supplementation in PBM diet groups (25 PBM+FPH, 50 PBM+FPH) resulted in similar CAT and G-6-PDH activities with the control diet than the non-supplemented diets. Moreover, dietary inclusion of PBM and PBM supplemented with FPH diets did not affect the intestinal morphology and haemato-biochemical responses of striped murrel. Thus, 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 digestive enzyme activities, intestinal morphology, antioxidant and metabolic enzyme activities and haemato-biochemical responses of striped murrel (*Channa striata*).

INTRODUCTION

Striped murrel (*Channa striata*) is distributed in many Southeast and South Asian countries (Hossain *et al*., 2008; Shafri and Abdul Manan, 2012). It is a commercially important high-value indigenous food fish with a high consumer preference, good flavor, meaty flesh with few intra-muscular bones, rich in amino acids and unsaturated fatty acids. Moreover, murrel has various medicinal properties, particularly wound healing and anti-inflammatory properties (Paripatananont, 2002; Lee *et al*., 2022). As a result, the world's striped murrel production has continuously increased over the last few decades, and it recorded 80,626 tons (live weight) in 2021 (FAO, 2023). Therefore, intensive farming of striped murrel has increased across the world in different rearing systems (Hung and Huy, 2007). In the intensive production of striped murrel, feed is the primary factor in determining the

* Corresponding author: sathish@tnfu.ac.in
0030-9923/2024/0001-0001 $ 9.00/0

production's success. Among the nutrients in the striped murrel diet, protein is the main component, accounting for half of the production cost of the diet. Fishmeal (FM) has been utilized as a prime source of protein in the striped murrel diet due to its high nutritional quality, digestibility, and palatability. However, due to decreased production and growing demand for FM, there is a need to find alternative feed ingredients that are inexpensive, locally accessible, and more cost-effective than fishmeal (FAO, 2022).

Poultry by-product meal (PBM) is an animal by-product obtained from poultry processing plants that contains viscera, head, bones, blood, feet, undeveloped eggs and feathers. The processed PBM has high protein (58-65%), protein digestibility (87-91%) (Bureau *et al*., 1999; Yu, 2004; Irm *et al*., 2020), appropriate levels of essential amino acids, fatty acids, vitamins, minerals and palatability factors (Cruz-Suárez *et al*., 2007). Therefore, PBM has emerged as one of the most alternate feedstuffs for FM. It has been evaluated in many carnivore fish species including striped murrel (Abdul-Halim *et al*., 2014), spotted rose snapper (Hernández *et al*., 2014), black sea bream (Irm *et al*., 2020), and barramundi (Siddik *et al*., 2019; Chaklader *et al*., 2021). However, a higher incorporation of PBM diets impaired the physiological development in several carnivore species due to the lack of some essential amino acids, fatty acids, and attractability in comparison to FM (Nengas *et al*., 1999; Cheng *et al*., 2002; Chaklader *et al*., 2020). In such situations, many researchers have proven that fish protein hydrolysate is used as a supplement to the low FM diet to improve the physiological activities of fishes like *Channa striata* (Suratip *et al*., 2023) and *Lates calcarifer* (Chaklader *et al*., 2020; Hong *et al*., 2021). Additionally, dietary components can affect digestibility capacities, antioxidant functions, and haemato-biochemical responses and may lead to a decrease in the animals' diet utilization efficiency and physiological responses.

Thus, the present investigation laid out to determine the effects of dietary substitution of FM protein with PBM and PBM supplementation with FPH on digestive enzyme activities, morphological structure of the intestine, antioxidant and metabolic status of liver and haemato-biochemical responses 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 acclimatized 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 lipid (Growel Feeds Pvt. Ltd., Andhra Pradesh, India) five times a day till satiation to reduce the cannibalistic activity. 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 experiment 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 by 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. The proximate and amino acid composition of feed ingredients for production of experimental diets are shown in Table I. 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 of PBM (25% and 50%) and supplemented with FPH. The control diet contained 35% 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. Briefly, all the dry feed ingredients were finely ground with a pulverizer, seized through 180 µm 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

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

Compositions	Feed ingredients							
	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.

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 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.

Digestive enzyme analysis

At the end of the feeding trial, the experimental fish intestine samples were collected from each replicate. The samples were washed with ice cold distilled water and homogenized (1:5, w/v) in phosphate buffer (pH 7.5). The homogenate samples were centrifuged at 4000 x *g* for 5 min at 4°C. After centrifugation, the supernatant was collected and stored at -20°C until the enzyme activities were analyzed. The total protein content of intestine samples was analyzed by Lowry *et al*. (1951) using bovine serum albumin as a standard. The amylase activities were analyzed using starch (1% w/v) as a substrate with the method of 3, 5-dinitrosalicylic acid (DNS) (Worthington, 1991). Maltose (0.3-5 µm/ml) was used to develop the standard curve to calculate the amylase activity of samples. The amylase activity was expressed as µmol of maltose per min per mg protein. The protease activity was analyzed using casein as a substrate (Drapeau, 1976). The protease activity was expressed as µmole tyrosine liberated per mg

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[4]	9	9	9	9	9
Corn gluten[5]	10	10	10	10	10
Wheat flour[4]	11	11	11	11	11
Broken rice[4]	6.2	6.43	6.46	6.65	6.68
Fish oil[1]	3	3	3	3	3
Palm oil[4]	2.2	1.2	0.2	1.2	0.2
Soy lecithin[6]	2	2	2	2	2
Di-calcium phosphate[7]	1	1	1	1	1
Vitamin mix[8]	1	1	1	1	1
Mineral mix[9]	1	1	1	1	1
Vitamin – C[7]	0.2	0.2	0.2	0.2	0.2
DL-methionine[10]	0.4	0.2	0.2	0.2	0.2
Binder[11]	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]SPAC Starch Products (India) Pvt Ltd., Erode, Tamil Nadu, India. [6]Otto chemie Pvt. Ltd., Mumbai, India. [7]Jain industrial chemicals, Chennai, India. [8]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. [9]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. [10]Evonik AG (DL-methionine: MetAMINO® – 99%). [11]PEGABIND®, Bentoli Agrinutrition India Pvt. Ltd., Chennai, India.

tissue protein per minute. The lipase activity was analyzed using para nitro phenyl palmitate (ρNPP) solution as a substrate (Winkler and Stuckman, 1979). The unit of lipase was expressed as enzyme unit (U) per mg protein.

Hepatic antioxidant and metabolic enzyme analysis

The hepatic catalase (CAT) enzyme activity was analyzed by measuring the decrease in concentration of H_2O_2 (Aebi, 1984). The hepatic superoxide dismutase (SOD) and glutathione peroxidase (GPx) were determined according to the method of Beauchamp and Fridovich (1971) and Wendel (1981), respectively. The hepatic metabolic enzymes such as malate dehydrogenase (MDH) and glucose-6-phosphate dehydrogenase (G-6-PD) were determined as described by Ochoa (1955) and Morales *et al*. (1990), respectively.

Intestine morphology analysis

At the end of the feeding trial, all the fish were starved for 24 h and three fish per treatment were euthanized using a high dose of tricaine methanesulfonate (MS-222, Sigma-Aldrich Inc.) to excise the intestine samples for histological analysis. The intestine samples were fixed in a 10% neutral buffered solution. Then, the samples were dehydrated with a series of ethanol concentrations, and the dehydrated samples were cleared in xylene and embedded in paraffin blocks according to standard histological procedure (Bell and Lightner, 1988). The blocks were then cut into slices (4−5 µm thickness) using an RM2125RTS rotary microtome (Leica), mounted onto glass slides, and stained with haematoxylin and eosin (H and E). Finally, the slides were observed using a DM750 light microscope (Leica), and the digital images were captured using a Leica EC3 camera and the Leica Application Suite Version 2.0.0.

Haemato-biochemical analysis

The blood samples were collected using a 1-ml heparinized syringe from the caudal vein of 3 anesthetized fish from each replicate. The collected blood samples were transferred to heparinized and non-heparinized tubes and stored on ice immediately. The heparinized blood samples were utilized to analyze the haematological parameters such as leucocyte (Leuk) counts, erythrocyte (Ery) counts, lymphocytes (%), haematocrit (HCT), haemoglobin (Hb), platelet (PLT) counts, mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC). The haematological parameters were analyzed using a fully automated haematology analyzer (Zybio Z3 Inc., China). The blood Nitroblue tetrazolium (NBT) or Respiratory burst activity assay was analysed using the protocol of Anderson and Siwicky (1995).

The non-heparinized blood samples were allowed

to clot at 4°C for 2 h until the serum was collected by centrifugation blood at 3500 g for 25-min at 4°C in a refrigerated centrifuge (Eppendorf Centrifuge 5804R). The serum biochemical parameters such as glucose (GLU), cholesterol (CHO), triglycerides (TG), total protein (TP), albumin (ALB), total bilirubin (T-BIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were analyzed by the commercial kit methods (Pathozyme Diagnostics Pvt. Ltd., Maharastra, India) using a semi-automatic biochemistry analyzer (Cytokine - SK3002B, Cytokine Healthcare Pvt Ltd., Chennai, Tamil Nadu, India). The serum total antiprotease activity was determined following the protocol of Zuo and Woo (1997). The lysozyme activity was measured using turbidimetrically according to the method of Sankaran and Gurnani (1972) using lyophilized hen egg white lysozyme (HEWL; Sigma) as standard.

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).

RESULTS

Digestive enzyme activities of striped murrel

The digestive enzyme activities such as amylase, protease, and lipase of striped murrel fed different levels of PBM and supplementation of FPH diets are shown in Table III. No significant differences ($p > 0.05$) were observed in amylase and lipase activities but a significant difference ($p < 0.05$) was observed in protease activities of striped murrel fed experimental diets. Significantly ($p < 0.05$) higher protease (7.03±0.38, 6.96±0.31 and 6.79±0.39 Umg^{-1} Protein) activities were recorded in fish fed 25 PBM+FPH, 50 PBM+FPH, and 35 FM (control) diets, respectively.

Hepatic antioxidant and metabolic enzyme activities of striped murrel

The hepatic antioxidant and metabolic enzyme activities such as CAT, SOD and GPx and G-6-PDH and MDH are shown in Table IV. No significant ($p > 0.05$) differences were observed in SOD, GPx and MDH activities of striped murrel fed experimental diets. Significantly ($p < 0.05$) higher CAT (49.26±5.16, 57.29±8.24 and 57.29±4.04 U mg^{-1} protein) and G-6-PDH (0.27±0.01, 0.28±0.01 and 0.27±0.02 U mg^{-1} protein) activities were found in fish fed 25 PBM+FPH, 50 PBM+FPH and 35 FM (control) diets than other diets, respectively.

Table III. Digestive enzyme activities (U mg^{-1} Protein) 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	
Amylase	2.97±0.35	3.06±0.45	2.90±0.23	2.80±0.32	2.93±0.21	0.901
Protease	6.79±0.39^{a}	6.41±0.28^{ab}	6.11±0.35^{b}	7.03±0.38^{a}	6.96±0.31^{a}	0.039
Lipase	3.96±0.29	3.65±0.21	3.74±0.21	3.77±0.10	3.88±0.14	0.386

The digestive enzyme activity 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 ($p < 0.05$).

Table IV. Antioxidant and metabolic enzyme activities (U mg^{-1} Protein) 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	
CAT	57.29±4.04^{a}	47.12±5.16^{b}	36.94±5.79^{b}	49.26±5.16^{a}	57.29±8.24^{a}	0.009
SOD	51.39±5.24	61.11±2.40	59.02±7.89	60.41±5.51	59.03±7.89	0.362
GPx	1.92±21	1.92±0.77	1.99±0.44	2.35±0.37	1.64±0.33	0.499
G-6-PDH	0.27±0.02^{a}	0.23±0.01^{b}	0.24±0.03^{b}	0.27±0.01^{a}	0.28±0.01^{a}	0.019
MDH	3.41±0.82	4.13±0.47	3.61±0.36	3.41±1.64	4.65±1.35	0.560

The antioxidant enzyme activity values are 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 ($p < 0.05$). CAT, Catalase; SOD, Superoxide dismutase; GPx, Glutathione peroxidase; G6PDH, Glucose-6-phosphate dehydrogenase; MDH, Malate dehydrogenase.

Table V. Intestinal morphology 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**	
Villi length (µm)	210.80±4.50^{a}	187.87±2.84^{b}	172.46±4.26^{c}	213.22±4.75^{a}	218.44±9.71^{a}	< 0.001
Villi width (µm)	32.89±1.90^{a}	28.05±00.62^{bc}	25.31±0.44^{c}	30.11±2.19^{ab}	32.41±3.05^{a}	0.003

The intestine villi length and width 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 ($p < 0.05$).

Table VI. Haemato-biochemical responses of striped murrel fed PBM with and without supplementation of FPH diets.

Haemato-biochemical parameters	**Experimental diets**					**p value**
	35 FM	**25 PBM**	**50 PBM**	**25 PBM + FPH**	**50 PBM + FPH**	
Haematological parameters						
Hb (g dl^{-1})	10.93±0.58	11.37±0.45	10.80±0.10	11.53±0.38	11.15±0.47	0.303
Leuk (1000/cumm)	22.93±1.69	24.19±1.25	23.17±1.62	23.68±0.89	23.41±1.16	0.815
Ery (million/cumm)	2.60±0.20	2.30±0.27	2.50±0.34	2.53±0.31	2.43±0.07	0.696
Ht (%)	42.47±0.57	42.60±0.65	42.90±0.56	42.60±0.92	42.93±0.25	0.857
MCV (fl)	164.02±13.52	186.34±20.98	173.78±23.96	169.51±16.62	176.78±5.18	0.610
MCH (pictograms)	25.75±1.55	26.68±0.85	25.17±0.10	26.08±1.73	26.86±0.75	0.425
MCHC (g dl^{-1})	42.26±4.69	49.63±4.54	43.76±6.10	44.38±6.98	47.48±1.64	0.442
NBT (OD at 450 nm)	1.05±0.06	1.01±0.07	0.98±0.02	1.06±0.05	1.06±0.06	0.381
Biochemical parameters						
GLU (mg dl^{-1})	44.73±1.06	42.62±1.41	42.54±2.86	43.70±1.65	43.89±2.06	0.610
TRY (mg dl^{-1})	243.58±2.38	240.03±2.05	241.00±1.26	243.48±1.22	241.52±2.87	0.219
CHO (mg dl^{-1})	105.08±3.73	103.65±2.83	105.14±2.42	105.69±2.37	104.55±1.82	0.907
TP (mg dl^{-1})	4.29±0.33	3.80±0.32	3.83±0.13	4.33±0.50	3.93±0.63	0.403
ALB (mg dl^{-1})	2.43±0.31	2.30±0.32	2.67±0.38	2.91±0.05	2.36±0.40	0.190
GLB (mg dl^{-1})	1.86±0.57	1.50±0.16	1.16±0.25	1.42±0.55	1.57±0.68	0.555
ALT (U ml^{-1})	29.02±5.47	28.26±4.18	28.02±2.86	28.44±5.27	26.99±2.72	0.983
AST (U ml^{-1})	31.45±0.80	32.19±1.08	31.59±0.83	32.62±0.16	32.23±0.49	0.340
ALP (U ml^{-1})	13.43±0.88	13.66±0.92	13.68±0.98	13.74±0.42	13.64±0.13	0.988
APA (%, Trypsin inhibition)	67.04±1.93	65.43±2.10	65.67±1.92	67.35±0.86	67.55±0.84	0.409
Lysozyme (µg/mL)	42.80±3.00	39.11±5.61	46.55±3.00	41.51±3.33	42.07±3.74	0.284

The Haemato-biochemical parameter 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 ($p < 0.05$). Hb, Haemoglobin; Leuk, Leukocytes; Ery, Erythrocytes; Ht, Haematocrit; MCV, Mean corpuscular volume; MCH, Mean corpuscular haemoglobin; MCHC, Mean corpuscular haemoglobin concentration; NBT, Nitroblue tetrazolium test; GLU, Glucose; TRY, Triglycerides; CHO, Cholesterol; TP, Total protein; ALB, Albumin, GLB, Globulin; ALT, Alanine transaminase; AST, Aspartate aminotransferase; ALP, Alkaline phosphatase; APA, Antiprotease activity.

Intestinal morphology of striped murrel

The intestinal villi length and width of striped murrel fed different levels of PBM and supplementation FPH diets are shown in Table V. Significant differences ($p < 0.05$) were observed in intestinal villi length and width of striped murrel fed with experimental diets. Higher villi length (210.80±4.50, 213.22±4.75 and 218.44±9.71 µm) was observed in fish fed 35 FM (control), 25 PBM+FPH and 50 PBM+FPH diets. Significantly higher villi width (32.89±1.90, 30.11±2.19 and 32.41±3.05 µm) was observed in fish fed 35 FM (control), 25 PBM+FPH and 50 PBM+FPH diets. However, fish fed 25 PBM+FPH (30.11±2.19 µm) group villi width was not significantly different with fish fed 25 PBM (28.05±00.62 µm) dietary group. The intestine morphology of striped murrel fed with different levels of PBM and supplementation of FPH diets are shown in Figure 1.

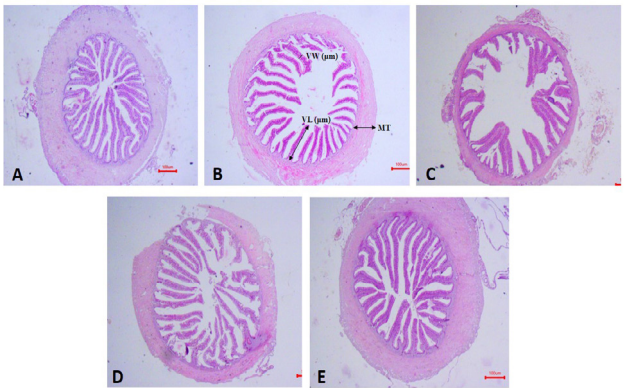

Fig. 1. Intestinal morphology (40 × magnification; scale bar with 100 µm) of striped murrel fed PBM with and without supplementation of FPH diets (A: 35 FM (control); B: 25 PBM; C: 50PBM; D: 25 PBM+FPH; E: 50 PBM+FPH). VL, Villi length; VW, Villi width and MT, Muscular thickness.

Haemato-biochemical responses of striped murrel

The haemato-biochemical responses of striped murrel fed different levels of PBM and supplementation of FPH diets are shown in Table VI. No significant differences ($p > 0.05$) were observed in haematology parameters such as Hb, Leuk, Ery, Ht, MCV, MCH, MCHC and NBT values of striped murrel fed different levels of PBM and supplementation of FPH diets. Similarly, no significant differences ($p > 0.05$) were observed in serum biochemical parameters (such as GLU, TRY, CHO, TP, ALB, GLB, ALT, AST, ALP, APA,) and Lysozyme values of striped murrel fed experimental diets.

DISCUSSION

The specific activity of digestive enzymes directly correlates with the apparent digestibility coefficient of diets. This present study showed that there were no major adverse effects on digestive enzyme activities in striped murrel when fish were fed PBM with or without supplementation of FPH diets. Likewise, no significant differences were observed in the protease and amylase activities of *Channa striata* when FM was replaced with up to 15% of FPH diets (Siddiah *et al*., 2022). Fontinha *et al*. (2021) showed that up to 83% of PBM had no effect on the protease, trypsin, chymotrypsin, lipase and amylase activities of *Sparus aurata*. Furthermore, the results are consistent with previous studies, such that a higher inclusion of PBM up to 50–55% does not alter the trypsin, chymotrypsin, lipase and amylase activities of Sobaity gilthead sea bream (*Sparidentex hasta*) (Hekmatpour *et al*., 2019) and *Sparus aurata* (Karapanogiotidis *et al*., 2019). However, the pepsin activity of *Sparus aurata* increased with increasing PBM inclusion. On the contrary, the activity of digestive enzymes was impaired and reduced when FM was replaced with more than 60% of the PBM in the diet of the great sturgeon (*Huso huso*) (Hassani *et al*., 2021).

The activity of the antioxidant enzyme displays the stress responses and the immune status of animals against oxidation. The enzymes, including CAT, SOD, and GPx, are intracellular antioxidant enzymes that scavenge the free radicals and reactive oxygen species (ROS) to reduce the harmful effects of oxidation. The SOD converts the superoxide ions (O_2^-) into hydrogen peroxide (H_2O_2) and then H_2O_2 catalyzed into water (H_2O), and molecular oxygen by CAT and GPx. In the present study, it is found that no significant differences were observed in SOD and GPx activities, and higher CAT activities were observed in fish fed with graded levels of PBM supplemented with FPH. These findings clarified that the partial replacement of FM protein with PBM and supplementation of FPH does not influence the immune responses and health status of striped murrel. Likely, similar levels of SOD activity was obtained in olive flounder fed with 50% chicken by-product meal (CBM) by replacing FM protein (Ha *et al*., 2021). Studies by Khosravi *et al*. (2015), Siddaiah *et al*. (2022) and Suratip *et al*. (2023) reported that on supplementation of fish-based protein hydrolyzates in low fish meal diets did not influence the antioxidant status of red sea bream (*Pagrus major*) and *Channa striata*. In accordance with the previous studies, no significant changes were observed in malate dehydrogenase (MDH) in *Channa striata* fed with PBM and supplementation of FPH diets by replacing FM. The hepatic metabolic enzymes G-6-PDH and MDH are involved in pentose phosphate and the citric acid cycle pathways. They might have produced metabolites such as 5-glucose-6-phosphate, oxaloacetate and NADPH, which are needed to synthesize nucleic acids, fatty acids, steroids, and certain amino acids and are also involved in antioxidant reactions by scavenging the free radicals, respectively (Rao and Rao, 1987; Takahashi-Íñiguez *et al*., 2016). Therefore, the results of PBM and FPH diets were not significant that of control and it is confirmed that using up to 50% of PBM supplemented with FPH can replace FM protein without affecting the metabolic activities of *Channa striata*.

The composition of feed ingredients significantly impacts intestinal structure, and the results of long intestinal folds and villi lengths reflect the overall performance of fish health and nutrient utilization efficiency of diets. In contrast, shorter folds and villi lengths are indications of poorer nutrient utilization and reduced growth responses. This study obtained significantly higher villi lengths and

heights in fish fed with 50% PBM and supplementation of FPH diets. Similar to our results, the surface area of the intestine of large yellow croaker (*Larimichthys crocea*) was not affected by FM replacement with up to 50% PBM (Wang *et al*., 2023). According to Siddik *et al*. (2019), FM replaced up to 70% of PBM without negatively impacting the intestines of Asian seabass (*Lates calcarifer*). However, total replacement of FM has significantly reduced the microvilli height and diameter of juvenile barramundi (*Lates calcarifer)* (Chaklader *et al*., 2020).

The haemato-biochemical responses are biomarkers to evaluate the effects of feed ingredients on the immunity and nutritional status of fish (Maita, 2007). In the present study, it was found that FM protein could be replaced upto 50% with PBM protein and FPH supplementation without affecting the haemato-biochemical responses of *Channa striata*. Similar results were found in haematological and biochemical responses of olive flounder (*Paralichthys olivaceus*), gilthead seabream (*Sparus aurata*), black sea bream (*Acanthoparus schlegelii*), and cobia (*Rachycentron canadum*) when fish fed with 50% to 60% of PBM incorporated diets, respectively (Zhou *et al*., 2011; Karapanagiotidis *et al*., 2019; Ha *et al*., 2021; Irm *et al*., 2020). A mixture of PBM, shrimp, and blood meal replaced with up to 80% of FM protein without affecting the serum biochemical parameters such as ALT, AST, CHO, TG, HDL-C (High-density lipoprotein cholesterol) and LDL-C (Low-density lipoprotein cholesterol) levels in juvenile hybrid grouper (Ye *et al*., 2019). Hekmatpour *et al*. (2019) have demonstrated that no significant alterations were observed in haemato-biochemical values except cholesterol and AST, and the values of ALT and ALP were significantly increased with the increment of PBM in diets of sobaity sea bream. The various earlier reports agreed with our results when the supplementation of FPH in low FM diet did not impact the immune responses of different fish species (Siddaiah *et al*., 2022; Suratip *et al*., 2023; Chaklader *et al*., 2021). No significant differences in serum NBT, APA, and lysozyme activities of *Channa striata* were observed and reported in this study. The serum NBT, APA, and lysozyme are components of the first-line defense mechanism and play a vital role in fish disease resistance and stress responses. Therefore, our present results confirmed that replacement of FM protein with PBM protein up to 50% and supplementation of FPH did not affect the non-specific immune responses of *Channa striata*. Similarly, 21.7% and 50% of CBM did not influence the lysozyme activities of olive flounder while replacing FM protein (Kim *et al*., 2021; Ha *et al*., 2021). Moreover, dietary FPH supplementation of a low FM diet has increased the innate immune responses of fish. Likewise, the low fish meal diet supplemented with shrimp, tuna and krill hydrolysate did not affect the serum NBT, antiprotease and lysozyme levels of red sea bream (Khosravi *et al*., 2015). Earlier studies like Siddaiah *et al*. (2022) and Suratip *et al*. (2023) have demonstrated that 10% FPH and 2.5% TH did not affect the lysozyme level and improved the innate immunity of *Channa striata*.

CONCLUSION

In conclusion, the present study revealed that PBM protein could replace up to 50% of FM protein when supplemented with FPH without affecting digestive enzyme activities, intestine morphology, antioxidant and metabolic enzyme activities and haemato-biochemical responses of *Channa striata* juveniles. Further studies are needed to achieve more than 50% FM replacement, which can be obtained with a diet containing PBM supplemented with FPH.

DECLARATIONS

Acknowledgements

The authors sincerely thank Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India for providing the facilities and grants to carry out this study.

Funding

This research received no specific grant from any funding agency.

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.

REFERENCES

Abdul-Halim, H.H., Aliyu-Paiko, M. and Hashim, R., 2014. Partial replacement of fish meal with poultry by-product meal in diets for snakehead, *Channa striata* (Bloch, 1793), fingerlings. *J. World Aquacult. Soc.*, **45**: 233-241. https://doi.org/10.1111/jwas.12112

Aebi, H., 1984. Catalase *in vitro*. *Methods Enzymol.*, **105**: 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3

Anderson, D. and Siwicki, A., 1995. *Basic hematology and serology for fish health programs.*

APHA, 2012. *Standard methods for the examination of*

water and waste water, 22nd edn. American Public Health Association., New York, USA.

Beauchamp, C. and Fridovich, I., 1971. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.*, **44**: 276-287. https://doi.org/10.1016/0003-2697(71)90370-8

Bell, T. and Lightner, D., 1988. A handbook of normal shrimp histology. *Special Publication No. 1. J. World Aquacult. Soc. Baton Rouge, LA,* **114**.

Bureau, D., Harris, A. and Cho, C., 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **180**: 345-358. https://doi.org/10.1016/S0044-8486(99)00210-0

Chaklader, M.R., Howieson, J., Siddik, M.A., Foysal, M.J. and Fotedar, R., 2021. Supplementation of tuna hydrolysate and insect larvae improves fishmeal replacement efficacy of poultry by-product in *Lates calcarifer* (Bloch, 1790) juveniles. *Sci. Rep.*, **11**: 4997. https://doi.org/10.1038/s41598-021-84660-5

Chaklader, M.R., Siddik, M.A. and Fotedar, R., 2020. Total replacement of fishmeal with poultry by-product meal affected the growth, muscle quality, histological structure, antioxidant capacity and immune response of juvenile barramundi, *Lates calcarifer*. *PLoS One*, **15**: e0242079. https://doi.org/10.1371/journal.pone.0242079

Cheng, Z.J., Behnke, K.C. and Dominy, W.G., 2002. Effects of poultry by-product meal as a substitute for fish meal in diets on growth and body composition of juvenile Pacific white shrimp, *Litopenaeus vannamei*. *J. appl. Aquacult.*, **12**: 71–83. https://doi.org/10.1300/J028v12n01_04

Cruz-Suárez, L.E., Nieto-López, M., Guajardo-Barbosa, C., Tapia-Salazar, M., Scholz, U. and Ricque-Marie, D., 2007. Replacement of fish meal with poultry by-product meal in practical diets for *Litopenaeus vannamei*, and digestibility of the tested ingredients and diets. *Aquaculture*, **272**: 466-476. https://doi.org/10.1016/j.aquaculture.2007.04.084

Drapeau, G.R., 1976. Protease from *Staphyloccus aureus*. *Methods Enzymol.*, **45**: 469–475. https://doi.org/10.1016/S0076-6879(76)45041-3

Duncan, D.B., 1955. Multiple range and multiple F-tests. *Biometrics*, **11**: 1-42. https://doi.org/10.2307/3001478

FAO, 2022. *The state of world fisheries and aquaculture 2018-meeting the sustainable development goals*. FAO.

FAO, 2023. Channa striata *Bloch, 1793*. Fisheries and Aquaculture Division [online]. Rome. https://www.fao.org/fishery/en/aqspecies/3062/en

Fontinha, F., Magalhães, R., Moutinho, S., Santos, R., Campos, P., Serra, C.R., Aires, T., Oliva-Teles, A. and Peres, H., 2021. Effect of dietary poultry meal and oil on growth, digestive capacity, and gut microbiota of gilthead seabream (*Sparus aurata*) juveniles. *Aquaculture*, **530**: 735879. https://doi.org/10.1016/j.aquaculture.2020.735879

Ha, M.S., Lee, K.W., Kim, J., Yun, A., Jeong, H.S., Lee, M.J., Baek, S.I., Cho, S.H., Kim, K.W., Lim, S.G., Lee, B.J., Hur, S.W., Son, M. and Lee, S., 2021. Dietary substitution effect of fish meal with chicken by-product meal on growth, feed utilization, body composition, haematology and non-specific immune responses of olive flounder (*Paralichthys olivaceus*). *Aquacult. Nutr.*, **27**: 315-326. https://doi.org/10.1111/anu.13176

Hekmatpour, F., Kochanian, P., Marammazi, J.G., Zakeri, M. and Mousavi, S.M., 2019. Changes in serum biochemical parameters and digestive enzyme activity of juvenile sobaity sea bream (*Sparidentex hasta*) in response to partial replacement of dietary fish meal with poultry by-product meal. *Fish Physiol. Biochem.*, **45**: 599-611. https://doi.org/10.1007/s10695-019-00619-4

Hernández, C., Sánchez-Gutiérrez, Y., Hardy, R., Benitez-Hernández, A., Domínguez-Jiménez, P., González-Rodríguez, B., Osuna-Osuna, L. and Tortoledo, O., 2014. The potential of pet-grade poultry by-product meal to replace fish meal in the diet of the juvenile spotted rose snapper *Lutjanus guttatus* (Steindachner, 1869). *Aquacult. Nutr.*, **20**: 623-631. https://doi.org/10.1111/anu.12122

Hong, Y.C., Chu, J.H., Kirby, R., Sheen, S.S. and Chien, A., 2021. The effects of replacing fish meal protein with a mixture of poultry by-product meal and fermented soybean meal on the growth performance and tissue nutritional composition of Asian seabass (*Lates calcarifer*). *Aquacult. Res.*, **52**: 4105-4115. https://doi.org/10.1111/are.15249

Hossain, M., Latifa, G. and Rahman, M., 2008. Observations on induced breeding of snakehead murrel, *Channa striatus* (Bloch, 1793). *Int. J. Sustain. Crop Prod.*, **3**: e8.

Hung, L. and Huy, H., 2007. Analysis of feeds and fertilizers for sustainable aquaculture development in Vietnam. *FAO Fisheries Tech. Pap.*, **497**: 331.

Irm, M., Taj, S., Jin, M., Luo, J., Andriamialinirina, H.J.T. and Zhou, Q., 2020. Effects of replacement of fish meal by poultry by-product meal on growth performance and gene expression involved in protein metabolism for juvenile black sea bream (*Acanthoparus schlegelii*). *Aquaculture*,

528: 735544. https://doi.org/10.1016/j.aquaculture.2020.735544

Karapanagiotidis, I.T., Psofakis, P., Mente, E., Malandrakis, E. and Golomazou, E., 2019. Effect of fishmeal replacement by poultry by-product meal on growth performance, proximate composition, digestive enzyme activity, haematological parameters and gene expression of gilthead seabream (*Sparus aurata*). *Aquacult. Nutr.*, **25**: 3-14. https://doi.org/10.1111/anu.12824

Khosravi, S., Bui, H.T.D., Rahimnejad, S., Herault, M., Fournier, V., Kim, S.S., Jeong, J.B. and Lee, K.J., 2015. Dietary supplementation of marine protein hydrolysates in fish-meal based diets for red sea bream (*Pagrus major*) and olive flounder (*Paralichthys olivaceus*). *Aquaculture*, **435**: 371-376. https://doi.org/10.1016/j.aquaculture.2014.10.019

Kim, J., Cho, S.H., Kim, T. and Hur, S.W., 2021. Substitution effect of fish meal with various sources of animal by-product meals in feed on growth, feed utilization, body composition, haematology and non-specific immune response of olive flounder (*Paralichthys olivaceus*, Temminck and Schlegel, 1846). *Aquacult. Res.*, **52**: 2802-2817. https://doi.org/10.1111/are.15132

Lee, V.L.L., Choo, B.K.M., Norazit, A., Noor, S.M. and Shaikh, M.F., 2022. *Channa striatus* in inflammatory conditions: A systematic review. *Front. Pharmacol.*, **13**: 1076143. https://doi.org/10.3389/fphar.2022.1076143

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. *J. biol. Chem.*, **193**: 256-275. https://doi.org/10.1016/S0021-9258(19)52451-6

Maita, M., 2007. Fish health assessment. In: *Dietary supplements for the health and quality of cultured fish* (eds. H. Nakagawa, M. Sato and D.M. Gatlin). CABI. p. 10e34. https://doi.org/10.1079/9781845931995.0010

Morales, A., García-Rejón, L. and De la Higuera, M., 1990. Influence of handling and/or anaesthesia on stress response in rainbow trout. Effects on liver primary metabolism. *Comp. Biochem. Physiol. A Physiol.*, **95**: 87-93. https://doi.org/10.1016/0300-9629(90)90014-J

Nengas, I., Alexis, M.N. and Davies, S.J., 1999. High inclusion levels of poultry meals and related by products in diets for gilthead seabream *Sparus aurata* L. *Aquaculture*, **179**: 13–23. https://doi.org/10.1016/S0044-8486(99)00148-9

Ochoa, S., 1955. Malic dehydrogenase and malic enzyme. *Methods Enzymol.*, **1**: 735-745. https://doi.org/10.1016/0076-6879(55)01128-2

Paripatananont, T., 2002. Snakehead and Pangasius catfish. In: *Nutrient requirements and feeding of finfish for aquaculture*. CABI Publishing Wallingford UK. pp. 396-401. https://doi.org/10.1079/9780851995199.0396

Rao, K.S. and Rao, K.V., 1987. The possible role of glucose-6-phosphate dehydrogenase in the detoxification of methyl parathion. *Toxicol. Lett.*, **39**: 211–214. https://doi.org/10.1016/0378-4274(87)90235-9

Sankaran, K. and Gurnani, S., 1972. On the variation in the catalytic activity of lysozyme in fishes. *Indian J. Biochem. Biophys.*, **9**: 162-165.

Hassani, S.M.H., Banavreh, A., Yousefi J.A., Mohseni, M., Shokri, M.M. and Rastekenari, Y.H., 2021. The feasibility of partial replacement fish meal with poultry by-products in practical diets of juvenile great sturgeon, *Huso huso*: Effects on growth performance, body composition, physiometabolic indices, digestibility and digestive enzymes. *Aquacult. Res.*, **52**: 3605-3616. https://doi.org/10.1111/are.15205

Shafri, M. and Manan, A.M., 2012. Therapeutic potential of the haruan (*Channa striatus*): From food to medicinal uses. *Malays. J. Nutr.*, **18**: 125-136.

Siddaiah, G.M., Kumar, R., Kumari, R., Damle, D.K., Rasal, K.D., Manohar, V., Sundaray, J.K. and Pillai, B.R., 2022. Dietary supplementation of fish protein hydrolysate improves growth, feed efficiency and immune response in freshwater carnivore fish, *Channa striata* fingerlings. *Aquacult. Res.*, **53**: 3401-3415. https://doi.org/10.1111/are.15848

Siddik, M.A., Chungu, P., Fotedar, R. and Howieson, J., 2019. Bioprocessed poultry by-product meals on growth, gut health and fatty acid synthesis of juvenile barramundi, *Lates calcarifer* (Bloch). *PLoS One*, 14(4): e0215025. https://doi.org/10.1371/journal.pone.0215025

Suratip, N., Charoenwattanasak, S., Klahan, R., Herault, M. and Yuangsoi, B., 2023. An investigation into the effects of using protein hydrolysate in low fish meal diets on growth performance, feed utilization and health status of snakehead fish (*Channa striata*) fingerling. *Aquacult. Rep.*, **30**: 101623. https://doi.org/10.1016/j.aqrep.2023.101623

Takahashi-Íñiguez, T., Aburto-Rodríguez, N., Vilchis-González, A.L. and Flores, M.E., 2016. Function, kinetic properties, crystallization, and regulation of microbial malate dehydrogenase. *J. Zhejiang Univ.*

Sci. B, **17**: 247–261. https://doi.org/10.1631/jzus.B1500219

Wang, X., Luo, H., Zheng, Y., Wang, D., Wang, Y., Zhang, W., Chen, Z., Chen, X. and Shao, J., 2023. Effects of poultry by-product meal replacing fish meal on growth performance, feed utilization, intestinal morphology and microbiota communities in juvenile large yellow croaker (*Larimichthys crocea*). *Aquacult. Rep.*, **30**: 101547. https://doi.org/10.1016/j.aqrep.2023.101547

Wendel, A., 1981. Glutathione peroxidase. *Methods Enzymol.*, **77**: 325–333. https://doi.org/10.1016/S0076-6879(81)77046-0

Winkler, U.K. and Stuckman, M., 1979. Glycogen, hyaluronate and some other polysaccharides greatly enhance the formation of exolipase by *Serratia marcescens*. *J. Bact.*, **138**: 663–670. https://doi.org/10.1128/jb.138.3.663-670.1979

Worthington, C.C., 1991. *Worthington enzyme manual enzymes and related biochemical*. Freehold, New Jersey, USA. pp. 212-215.

Ye, H., Zhou, Y., Su, N., Wang, A., Tan, X., Sun, Z., Zou, C., Liu, Q. and Ye, C., 2019. Effects of replacing fish meal with rendered animal protein blend on growth performance, hepatic steatosis and immune status in hybrid grouper (*Epinephelus fuscoguttatus*♀ × *Epinephelus lanceolatus*♂). *Aquaculture*, **511**: 734203. https://doi.org/10.1016/j.aquaculture.2019.734203

Yu, Y., 2004. Replacement of fishmeal with poultry byproduct meal and meat and bone meal in shrimp, tilapia and trout diets. *Avances Nutr. Acuicola*., https://nutricionacuicola.uanl.mx/index.php/acu/article/view/194

Zhou, Q.C., Zhao, J., Li, P., Wang, H.L. and Wang, L.G., 2011. Evaluation of poultry by product meal in commercial diets for juvenile cobia (*Rachycentron canadum*). *Aquaculture*, **322**: 122–127. https://doi.org/10.1016/j.aquaculture.2011.09.042

Zuo, X. and Woo, P., 1997. Natural anti-proteases in rainbow trout, *Oncorhynchus mykiss* and brook charr, *Salvelinus fontinalis* and the *in vitro* neutralization of fish α2-macroglobulin by the metalloprotease from the pathogenic haemoflagellate, *Cryptobia salmositica*. *Parasitology,* **114**: 375-382. https://doi.org/10.1017/S0031182096008578