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Impact of Plant-based Protein Substitutes on Growth Performance, Histology and Gene Expression in Striped Snakehead, *Channa striata* (Bloch, 1793) Fingerlings

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ABSTRACT

Background: This research assessed the effects of incorporating various plant-based protein sources, including corn gluten meal (CGM), cottonseed meal (CSM), groundnut meal (GNM) and mustard seed meal (MSM), alongside soybean meal (SBM) in the diet of juvenile snakehead (*Channa striata*). Building on our previous findings that established an optimal SBM inclusion level of 25%, this subsequent trial explored the addition of these plant protein ingredients at a 10% inclusion rate, supplemented by SBM.

Methods: The experimental diets were designed to be isonitrogenous (42% crude protein), isolipidic (9% crude fat) and isoenergetic (16.1-16.2 MJ/kg). The trial was conducted over eight weeks with 400 healthy murrel fingerlings (average weight 27.0±0.06 g), sourced from Growel Feeds Pvt Ltd, a commercial fish hatchery in Andhra Pradesh. The fingerlings were reared in fiberglass reinforced plastic (FRP) tanks (150 L capacity, with 20 fish per tank).

Result: Evaluations of growth performance, haematological parameters, histological analyses and gene expression showed no significant differences among the various experimental groups. These results suggest that all the plant-based protein ingredients tested provided comparable growth performance and can be effectively used as alternative protein sources in conjunction with SBM for *Channa striata* feed formulations. This study highlights the potential for integrating cost-effective, plant-based protein sources into the feed for striped murrel fingerlings, promoting sustainability and economic viability.

Key words: *Channa striata*, Gene expression, Plant protein, Soybean meal.

INTRODUCTION

The striped murrel (*Channa striata*) is a rapidly growing and versatile carnivorous fish found in Southeast and Southern Asia, prized for its strong market demand (Gustiano *et al*., 2021; Kumar *et al*., 2022). Its commercial cultivation has played a significant role in advancing global aquaculture (FAO, 2020). Being a carnivorous species, *C. striata* necessitates high-protein diets, which are conventionally supplied by fishmeal (FM). However, this reliance raises sustainability concerns due to limited availability and fluctuating prices (Vo *et al*., 2015; Glencross *et al*., 2024). Consequently, there is a growing interest in alternative protein sources, such as plant proteins, that can replace or supplement fishmeal while ensuring adequate growth performance (Maksimenko *et al*., 2024).

Plant proteins, including soybean meal (SBM), are economically advantageous and contribute to the expansion of aquaculture; yet, their use can be restricted by anti-nutritional factors and lower digestibility in carnivorous fish (NRC, 2011; Kumari *et al*., 2013). Addressing these issues requires the development of optimized feed formulations (Glencross *et al*., 2024). Techniques such as fermentation and enzymatic treatment may enhance the quality of plant proteins (Maksimenko *et al*., 2024).

This study aims to assess the supplementation of SBM with CGM, CSM, GNM and MSM in *C. striata* diets to foster sustainable and cost-effective aquaculture practices.

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Degossypolized cottonseed protein (DCP) is produced by the solvent extraction of water-soluble carbohydrates and free gossypol from cottonseed meal, which finally contains high-quality protein and a low concentration of free gossypol (Wang *et al*., 2019). Detection of aflatoxins in corn gluten meal and groundnut meal is carried out by high-performance thin-layer Chromatography based on their fluorescence under UV light with an acceptable level below 20 µg kg^{-1} (Jaiswar *et al*., 2022).

MATERIALS AND METHODS

Ethical statement

The experiment was conducted following the procedures of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment and Forests (Animal Welfare Division), Govt. of India on care and use of animals in scientific research. This study was approved by the ethical committee of Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India. The experiment was conducted for 60 days at Growel Fish Hatchery, Perikegudem, Andhra Pradesh, India.

Experimental diets

Five distinct diets were formulated to evaluate the effects of alternative plant protein sources, including Corn Gluten Meal (CGM), Cottonseed Meal (CSM), Groundnut Meal (GNM) and Mustard Seed Meal (MSM), alongside soybean meal (SBM) for *Channa striata*. The diets comprised a control group with only SBM and four additional groups featuring CGM (T2), CSM (T3), GNM (T4) and MSM (T5). All diets were designed to be isonitrogenous, isolipidic and isoenergetic, containing 42% crude protein, 9% crude fat and gross energy levels of 16.1-16.2 MJ/kg. The feed was processed into floating pellets of 1.2 mm in diameter using a twin-screw extruder. A detailed composition can be found in Table 1.

Fish husbandry

Juvenile *Channa striata* were acquired from Growel Feeds Hatchery located in Andhra Pradesh and were acclimatized for a duration of 20 days on a commercial diet (42% CP, 9% CL). The feeding trial was executed in a Recirculating Aquaculture System (RAS) utilizing 20 fiberglass-reinforced plastic (FRP) tanks, each with a capacity of 150 L and supplied with sand-filtered water, aeration and covered with fiber mats. The FRP tanks were linked to biofiltration units, with water recirculated four times per day.

A total of 400 juveniles (average weight 27.0±0.06 g) were randomly allocated into the 20 tanks (20 fish per tank). Fishes were hand fed their respective diets till apparent satiety three times a day (8:00, 12:00 and 17:00 h) at 10% body weight until satiation for 8 weeks. Cannibalism cannot be completely eliminated in snakehead culture, but maintaining size uniformity and ensuring satiation feeding effectively minimized cannibalistic interactions in the experimental setup. Initial body composition analyses were conducted by sampling three fish per tank, while final body

Table 1: Feed formulation and proximate composition of experimental diets ($g.kg^{-1}$ diet).

Ingredients	Control (SBM)	SBM+CGM	SBM+CSM	SBM+GNM	SBM+MSM
Fish meal	29.0	29.0	29.0	29.0	29.0
Soybean meal	25.0	25.0	25.0	25.0	25.0
Poultry By-product meal	14.6	3.1	6.7	5.8	9.3
Corn Gluten meal	0.0	10.0	0.0	0.0	0.0
Cottonseed meal	0.0	0.0	10.0	0.0	0.0
Groundnut meal	0.0	0.0	0.0	10.0	0.0
Mustard seed meal	0.0	0.0	0.0	0.0	10.0
Rice	27.6	27.1	24.1	24.5	22.0
Soy lecithin	0.5	0.5	0.5	0.5	0.5
Fish oil	1.0	1.0	1.0	1.0	1.0
Ricebran oil	1.4	3.1	2.8	3.1	2.3
MCP	0.0	0.0	0.0	0.0	0.0
Salt	0.5	0.5	0.5	0.5	0.5
Lysine Hcl	0.0	0.3	0.0	0.0	0.0
DL methionine	0.1	0.0	0.1	0.1	0.1
L threonine	0.0	0.1	0.0	0.1	0.0
Vitamin mixture[a]	0.2	0.2	0.2	0.2	0.2
Mineral mixture[b]	0.2	0.2	0.2	0.2	0.2
Total	100.00	100.00	100.00	100.00	100.00
Proximate composition (% DM basis)					
Moisture	7.32	7.64	6.38	6.6	9.19
Crude protein	42.02	43.49	42.9	42.52	41.42
Fat	10.18	10.91	9.5	9.88	11
Fibre	1.6	1.7	1.9	2.2	1.9
Ash	8.54	7.92	8.94	8.85	8.8

[a] Contain the following per kg of vitamin premix: Thiamine, 10 g, Riboflavin, 7.5 g, Pyridoxine, 10 g, VB12, 0.01 g, VB5, 32.5 g, VK, 12.5 g, VE, 60 g, VD3, 1250000 IU, Niacin, 40 g, Biotin 0.4 g, Folic acid, 4 g, VA, 2750000 IU, C-Stable form, 125 g.

[b] Contain the following per kg of mineral premix: $CuSO_4 \cdot 5H_2O$, 5 g, $CoCl_2 \cdot 7H_2O$, 0.025 g, KI, 0.03 g, MnSO„ ·H, O , 10.00 g, Iron, 15 g, Selenium, 0.15 g, Iodine, 2.5 g, Zinc, 30 g.

composition was assessed at the conclusion of the study. Water quality parameters such as temperature (26.1±0.5°C), dissolved oxygen (5.5±0.1 ppm), pH (7.5±0.1) and ammonia levels (0.02±0.006 ppm) were monitored twice daily, adhering to APHA (2005) standards.

Sampling methods and calculations

Following the eight-week feeding period, fish were deprived of food for 24 hours, weighed collectively. The fishes were anesthetized with clove oil (50 µl/L) and three fish per tank were selected for measuring length, body weight, visceral weight and liver weight to compute the condition factor (K), viscero-somatic index (VSI) and hepato-somatic index (HSI). For blood analysis, blood samples were collected from the caudal vein into EDTA-coated tubes. Plasma was obtained via centrifugation (3000 × g for 5 minutes) and subsequently stored at -20°C. Muscle samples and foregut sections were also collected for gene expression and histological assessments. Bio-growth parameters were calculated using standard formulas.

Analytical chemistry

Proximate analysis for crude lipid, crude protein, moisture, dry matter, ash and energy content in both diets and fish samples was performed following the AOAC (2000) guidelines. Moisture content was determined by drying samples at 105°C until a constant weight was reached. Crude protein levels were ascertained via nitrogen content using the Kjeltec system, while crude lipid was assessed through petroleum ether extraction using the Soxtec system. Crude fiber was analyzed using acid-alkali digestion with the Fibretech system and ash content was determined by incineration at 550°C for six hours.

Antioxidant enzyme analysis

Plasma concentrations of superoxide dismutase (SOD) and catalase (CAT) were measured. SOD activity was evaluated by tracking the increase in absorbance of Epinephrine at 480 nm, expressed as U/mg protein (Marklund and Marklund, 1974). Catalase activity was assessed by monitoring the decrease in H_2O_2 absorbance at 240 nm, expressed as ìmol/mg protein/min (Aebi, 1984). Protein concentration in serum was determined using AOAC's method (1951).

Haematological and biochemical parameters

Three fish per tank were anesthetized with clove oil (50 µLL) for blood sampling from the caudal vein using EDTA-treated syringes. Haematological parameters (RBC, WBC, haematocrit, MCV, MCH, MCHC) were analyzed using an automatic analyzer (Cell Tech 380, India). Biochemical markers (glucose, serum cholesterol, triglycerides) were evaluated using a semi-automatic biochemical analyzer.

Histological analysis

Foregut samples were fixed in Davidson's fixative, then dehydrated in ethanol and embedded in paraffin. Sections (7 µm) were stained with haematoxylin and eosin. Measurements of villus height, thickness and muscular thickness were conducted using image analysis software. This work was performed at Madras Veterinary College, Vepery, Tamil Nadu.

Quantitative Real-time PCR (qRT-PCR)

Muscle samples from three fish per tank were collected for gene expression analysis of *MyoD* and *MyoG*. RNA extraction was performed using RNA iso-plus (Takara Bio) and cDNA synthesis was conducted with a first-strand synthesis kit (Thermo Scientific). The list of primers given in Table 2. Gene expression was quantified through qRT-PCR on a CFX96 system (Bio-Rad) under specific cycling conditions. Beta-actin was used as the housekeeping gene and relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The study was carried out at the State Referral Laboratory for Aquatic Animal Health, Dr. M.G.R. Fisheries College and Research Institute, Madhavaram, Tamil Nadu.

Statistical analysis

Data were expressed as mean±standard deviation (SD) across four replicates. Statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test ($p<0.05$). All statistical analyses were conducted using SPSS software version 20.0 (IBM), with variations analyzed at $p<0.05$ levels. The quantitative results were presented as mean±SD.

RESULTS AND DISCUSSION

Growth performance, feed utilization and somatic parameters

There were no significant differences in final weight (FW), weight gain (WG), specific growth rate (SGR), or feed conversion ratio (FCR) among the various treatment groups, which included both the SBM control and the diets supplemented with plant proteins. The protein efficiency ratio (PER) and SGR remained consistent across all dietary treatments. Furthermore, the addition of plant proteins (CGM, CSM, GNM, MSM) did not significantly influence the

Table 2: Primers used for qRT-PCR analysis of selected genes for *Channa striata* fed experimental diets.

Gene name	Gen bank number	Primer sequence (5´-3´)
Myogenic factor (*MyoD*)	GU246722	Forward: CCACCTGTCAGACAACCAGA
		Reverse: ACTGCGTTCGCTCTTCAGAC
Myogenin (*MyoG*)	GU246725	Forward: CTCAACCAGCAGGACACTGA
		Reverse: ATCCTCGCTGCTGTAGCTCT
β-actin	EU887951.1	Forward: CCACACAGTGCCCATCTACGA
		Reverse: CCACGCTCTGTCAGGATCTTCA

viscerosomatic index (VSI), hepatosomatic index (HSI), survival rates, or condition factor (p>0.05) given in Table 3.

Whole body composition

Proximate analysis of whole body composition indicated no significant differences across the different diet treatments (p>0.05) given in Table 4. All evaluated parameters were stable, suggesting that variations in diet did not affect overall body composition.

Intestinal morphology

The inclusion of alternative plant proteins did not result in significant changes in villi length. However, a notable reduction in villi width was observed in these treatment groups when compared to the control group, provided in Table 5. Despite this decrease in villi width, the structural integrity of the intestine, as measured by muscular thickness, remained consistent across all treatment groups.

Haematological and biochemical indices

No significant alterations were noted in haematological parameters (RBC, WBC, haemoglobin, MCV, MCH) or biochemical indicators (albumin, globulin, total protein) among the treatment groups. The addition of plant proteins (CGM, CSM, GNM, MSM) did not adversely affect these health indicators, thus ensuring the well-being of the fish presented in Table 6.

Antioxidant enzymes

The Nitroblue Tetrazolium (NBT) assay revealed no significant differences in phagocyte activity. Additionally, the activities of catalase (CAT) and superoxide dismutase

Table 3: Growth performance and survival of *C. striata* fingerlings fed different experimental diets after 8 weeks of feeding trial.

	Control (SBM)	SBM+CGM	SBM+CSM	SBM+GNM	SBM+MSM	*p*- value
IW (g)	27.05±0.04	27.12±0.08	27.01±0.12	27.06±0.05	27.04±0.07	0.985
FW (g)	55.51±5.92	54.05±3.99	58.79±1.45	54.19±3.28	54.15±4.71	0.925
WG (g)	28.51±5.98	27.05±406	25.79±1.30	27.19±3.12	27.15±4.68	0.925
WG%	105.64±22.42	100.22±15.34	95.49±4.30	100.66±11.03	100.55±17.18	0.923
FCR	1.89±0.21	2.10±0.33	1.91±0.17	2.04±0.21	1.96±0.21	0.705
SGR (%/day)	1.28±0.19	1.23±0.13	0.19±0.04	1.24±0.10	1.24±0.15	0.937
PER	1.33±0.15	1.22±0.23	1.32±0.12	1.24±0.13	1.28±0.15	0.814
HSI (%)	1.81±0.21	1.78±0.25	1.65±0.22	1.57±0.19	1.65±0.14	0.467
VSI (%)	7.46±0.68	7.23±0.86	7.08±0.93	7.20±0.13	7.47±0.98	0.94
Survival (%)	96.25±4.79	91.25±2.50	97.50±5.00	93.75±2.50	96.25±2.50	0.167
Condition factor	0.85±0.04	0.89±0.09	0.87±0.06	0.94±0.04	0.93±0.07	0.297

Abbreviations: IBW- Initial body weight; FBW- Final body weight (g); WG- Weight gain (g); FCR- Feed conversion ratio; FER-Feed efficiency ratio; DGR- Daily growth rate; SGR- Specific growth rate (%); PER- Protein efficiency ratio; WG- Weight gain; HSI- Hepatosomatic index; VSI- Viscerosomatic index.

Note: Values were expressed as means±SD of quadruplicate tanks per treatment (n=3) and values with different superscripts indicate significant differences as determined by Duncan test (p<0.05).

Table 4: Chemical composition of the whole body of *C. striata* fingerlings fed different experimental diets before and after feeding trial (% wet weight basis).

Proximate	Control (SBM)	SBM+CGM	SBM+CSM	SBM+GNM	SBM+MSM
Moisture	9.42±0.43	9.77±0.34	9.65±0.45	9.55±0.42	9.85±0.31
Protein	55.4±0.71	53.45±1.23	54.67±1.40	54.97±1.81	55.20±1.19
Fat	24.6±1.34	26.8±1.19	24.25±1.71	24.6±1.79	24.62±1.78
Ash	10.2±0.98	9.67±0.41	10.90±0.75	10.25±0.70	10.12±0.94
Silica	0.23±0.03	0.24±0.04	0.29±0.02	0.25±0.02	0.26±0.02

Note: Values are presented as mean ± SD (*n* = 3); mean values in the same row with different superscript letters differ significantly (p<0.05).

Table 5: Histological parameters of fore-gut cross sections of juvenile *Channa striata* at the end of the 8 week feeding trial.

Parameters	Control (SBM)	SBM+CGM	SBM+CSM	SBM+GNM	SBM+MSM
Villi length (µm)	325.22±13.28	329.13±3.89	315.50±5.13	350.14±22.57	304.10±47.08
Villi width (µm)	37.53±2.61^{b}	27.08±2.47^{a}	27.98±3.36^{a}	29.91±1.53^{a}	31.32±2.59^{a}
Muscularis thickness (µm)	47.51±8.03	60.35±7.67	60.71±8.99	48.84±16.87	58.75±11.12

Value are means of 10 observations of three fish from each of four replicate tanks.

(SOD) showed no significant changes in serum and liver samples, tabulated in Table 7. There were also no significant variations in albumin, globulin, or total protein levels, indicating stable protein quality and metabolic health.

Relative mRNA expression

No significant increase in the expression of *MyoD* and *MyoG* mRNA was detected in fish that were fed diets containing alternative plant proteins (CGM, CSM, GNM, MSM). This finding suggests that these plant protein sources did not influence the transcription levels of myogenic regulatory factors when compared to the SBM control diet.

The current study focused on evaluating the incorporation of various plant protein sources, including CGM, CSM, GNM and MSM, in the feed formulations for snakehead (*Channa spp*.). The findings revealed no significant differences in growth performance, feed conversion ratio (FCR), or weight gain, suggesting that these ingredients can effectively substitute fishmeal, consistent with previous research (Liu *et al*., 2023; Fei *et al*., 2024) (Fig 1). There was no

Table 6: Changes of haematological and biochemical indices in *C. striata* fingerlings fed different experimental diets after feeding trial.

Index	Control (SBM)	SBM+CGM	SBM+CSM	SBM+GNM	SBM+MSM
Glucose (mg/dL)	135.75±72.43	112.25±66.54	109.25±50.77	88.75±40.59	68.50±13.07
Hb (g/dL)	11.47±1.13	10.45±1.39	9.37±2.72	9.75±1.07	11.00±0.48
RBC (×106/mm3)	3.08±0.37	3.04±0.31	2.77±0.58	3.06±0.55	3.03±0.19
WBC (×103/mm3)	1.73±0.70	1.59±0.14	1.43±0.31	1.68±0.23	1.77±0.06
Total cholesterol (mg/dL)	92.00±11.57	80.25±17.58	89.75±26.05	208.00±262.01	87.00±10.92
VLDL (mg/dL)	35.75±18.57	35.5±2.64	34.25±10.21	25.00±8.60	35.25±17.03
Triglycerides (mg/dL)	180.50±91.40	168.75±14.5	309.00±274.20	127.50±44.06	177.75±86.31
HDL	18.00±2.30	15.5±3.69	17.5±5.44	14.75±0.96	17.00±2.45
Total protein (mg/dL)	5.07±0.74	5.10±0.81	4.97±0.77	4.65±0.99	4.25±0.88
Albumin (mg/dL)	3.2±0.29	2.97±0.61	2.85±0.43	2.78±0.67	2.57±0.51
Globulin (mg/dL)	1.87±0.54	2.12±0.40	2.12±0.51	1.87±0.33	1.67±0.38

Note: Values are means ± SD (n=3). The various superscript in the same row denotes significant differences among groups ($p<0.05$).

Table 7: Effects of different inclusion of alternative plant protein ingredients along with SBM on antioxidant enzymes activity in serum of juvenile *Channa striata.*

Dietary group	Catalase serum (U/mg of protein)	Catalase liver (U/mg of protein)	SOD serum (U/mg of protein)	SOD liver (U/mg of protein)	NBT
Control (SBM)	2.49±1.10	10.19±5.39	12.56±1.96	8.32±1.29	0.71±0.06
SBM+CGM	1.86±0.64	14.09±4.52	11.89±2.00	7.05±1.99	0.62±0.07
SBM+CSM	1.83±0.84	5.73±1.91	12.73±2.21	8.01±4.91	0.57±0.08
SBM+GNM	2.26±1.05	10.18±2.45	13.63±2.84	6.47±0.98	0.44±0.04
SBM+MSM	2.78±1.34	9.21±3.20	15.00±2.90	6.93±0.78	0.44±0.03

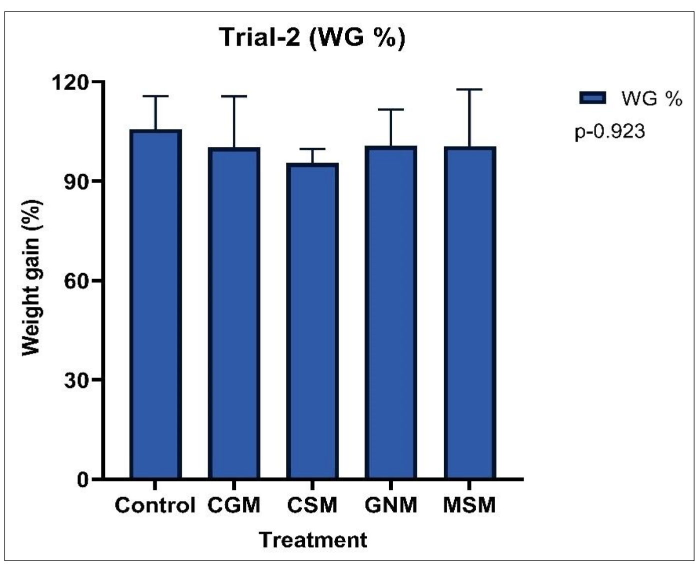

Fig 1: Weight gain (%) of *C. striata* fingerlings fed different experimental diets after 8 weeks of feeding trial (mean±SD; n = 3).

statistically significant difference found in the length-weight relationship of sea bream fed diets with varying protein levels (Korkut *et al*., 2018). The plant proteins demonstrated their potential as sustainable alternatives, showing no detrimental impact on growth or feed efficiency, in alignment with studies conducted on other fish species (Zhang *et al*., 2014; Watanabe and Pongmaneerat, 1993; Fan *et al*., 2023). In the study by Priyatharshni *et al*., 2023 replacement of soybean meal study has also been evaluated with sesame meal in Tilapia fishes.

Proximate body composition analysis indicated no significant variations between the fish fed SBM alone and those supplemented with plant proteins, confirming that these alternative proteins did not compromise the nutritional quality of the fish (Love, 1980; Saliu *et al*., 2017; Zhang *et al*., 2014). Histological evaluations showed healthy intestinal tissue with no notable differences across diets, contrasting with earlier studies that reported negative impacts on intestinal morphology at high SBM levels (Zhang *et al*., 2018; Wang *et al*., 2017) (Fig 2).

Using a Plant Protein blend of Wheat Meal, Wheat Gluten and Soybean Meal in rainbow trout diet was found faesible without apparent decrement in growth or intestinal health status (Calabria *et al*., 2021). The investigation of Madhubabu *et al*., 2021 demonstrated that a combination of plant protein sources could substitute 50% of fishmeal

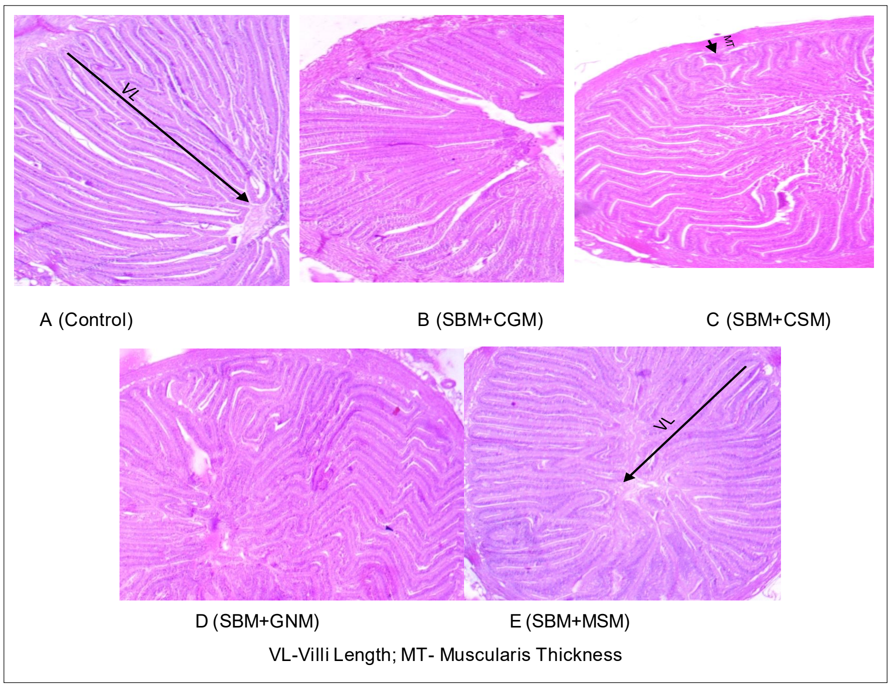

Fig 2: Histological comparison of intestinal differences of snakehead fish in the control (A), SBM+CGM (B), SBM+CSM (C), SBM+GNM (D) and SBM+MSM (E). The magnification was 10x.

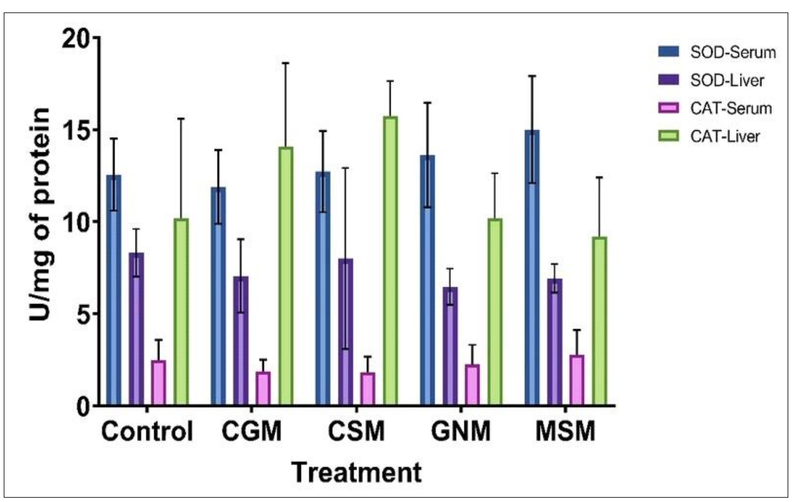

Fig 3: SOD and CAT activity of *C. striata* fingerlings fed different experimental diets after 8 weeks of feeding trial (mean±SD; n =3).

in the diet of Asian seabass without having any adverse effect on growth performance.

The antioxidant enzymes, SOD and CAT, are vital for the immune function of fish. Although some studies have suggested that plant protein inclusion may reduce SOD activity (Huang *et al*., 2023), this study found no significant differences regarding antioxidant activity or respiratory burst activity (RBA) (Fig 3). In the treatment with GNM, cholesterol levels were noted comparatively high, that implies with the study of Xie *et al*., 2025 where SBM diet with cholesterol supplementation could increase the activities of intestinal enzymes (*e.g.*, amylase) and restore the structural integrity of the intestinal lining, including villus height and goblet cell count. Additionally, no notable changes in serum biochemical indices (total protein, albumin, glucose) were observed, affirming that the inclusion of plant proteins did not adversely affect immune or metabolic health, in line with findings in rainbow trout and snakehead (Nazir *et al*., 2020; Suratip *et al*., 2023). Das *et al*., 2015 has evaluated cost effective floating feeds by replacement of soybean meal with alternative feed ingredients. Also net profit with respect to total biomass harvested and feed cost reduction was also recorded in diets using alternative plant protein ingredients like CGM, CSM, GNM and MSM.

Regarding muscle growth, the lack of significant upregulation of *MyoD* and *MyoG* gene expression suggests that plant protein supplementation does not negatively impact muscle development, supporting the feasibility of these ingredients for aquafeed. Overall, these results highlight the viability of using alternative plant proteins in snakehead diets without compromising growth, immunity, or tissue health.

CONCLUSION

This study showed that plant protein ingredients such as Corn gluten meal (CGM), cottonseed meal (CSM), Groundnut meal (GNM) and Mustard meal (MSM), when combined with soybean meal (SBM), supported comparable growth performance in *Channa striata* at a 10% inclusion level. The lack of significant differences suggests these alternatives can partially replace traditional protein sources without compromising fish performance. Future research should explore higher inclusion levels of these plant proteins to identify optimal formulations that support sustainability and cost-effectiveness in aquafeed development. This may provide insights into incorporating higher levels without negatively affecting growth or feed conversion efficiency. Histological and gene expression analyses showed no adverse effects, highlighting the potential for integrating these plant proteins into future snakehead feed formulations.

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Conflict of interest

All authors declare that they have no conflict of interest.

REFERENCES

Aebi, H. (1984). Catalase *in vitro*. In Methods in enzymology. *Academic Press*. **105:** 121-126.

AOAC, Association of Official Analytical Chemists. (2000). Official Methods of Analysis. Vol. II, 17th Edition, Washington DC.

APHA (2005). Standard Methods for the Examination of Water and Wastewater. 21st Edition, American Public Health Association American Water Works Association Water Environment Federation, Washington DC.

Calabria, V.G., Peñaranda, D.S., Jover-Cerdá, M., Llorens, S.M. and Tomás-Vidal, A. (2021). Successful inclusion of high vegetable protein sources in feed for rainbow trout without Decrement in Intestinal Health. *Animals*. **16;11(12):** 3577. doi: 10.3390/ani11123577.

Das, K.C., Toppo, S., Mohanty, T., Pradhan, C., Mohanta, K.N., Giri, S.S. (2015). Cost effective floating feeds for Indian Major Carps (IMC) by replacement of soybean meal with alternative feed ingredients. *Indian Journal of Animal Research*. **50(4):** 526-528. doi: 10.18805/ijar.7090.

Fan, Z., Cheng, M., Wang, L., Li, C., Wu, D., Li, J., Cheng, Z., Zhang, H. and Li, W. (2023). Feasibility evaluation of fermented peanut meal to replace soybean meal in the diet of common carp (*Cyprinus carpio*): Growth performance, serum biochemistry, intestinal health and microflora composition. Aquaculture reports (31).

FAO. (2020). Fishery and Aquaculture Statistics - Yearbook. Rome. https://doi.org/10.4060/cc7493en.

Fei, S., Kang, J., Ou, M., Liu, H., Zhang, X., Luo, Q., Li, K., Chen, K. and Zhao, J. (2024). Effects of essential amino acids supplementation in a low-protein diet on growth performance, intestinal health and microbiota of juvenile blotched snakehead (*Channa maculata*). Fish and Shellfish Immunology. **149:** 109555. https://doi.org/10.1016/j.fsi.2024.109555.

Glencross, B., Ling, X., Gatlin, D., Kaushik, S., Overland, M., Newton, R. and Valente, L.M.P. (2024). A SWOT analysis of the use of marine, grain, terrestrial-animal and novel protein ingredients in aquaculture feeds. *Reviews in Fisheries Science and Aquaculture*. **32(3):** 396-434. https://doi.org/10.1080/23308249.2024.2315049.

Gustiano, R., Kurniawan, K. and Haryono, H. (2021). Optimizing the utilization of genetic resources of Indonesian native freshwater fish. *Asian Journal of Conservation Biology*. **10(2).** https://doi.org/10.53562/ajcb.67022

Huang, H., Li, X., Cao, K. and Leng, X. (2023). Effects of replacing fishmeal with the mixture of cottonseed protein concentrate and clostridium autoethanogenum protein on the growth, nutrient utilization, serum biochemical indices, intestinal and hepatopancreas histology of rainbow trout (*Oncorhynchus mykiss*). *Animals*. **13(5):** 817.

Jaiswar, R., Sarathchandra, G., Shanmugam, S.A., Felix, N. and Narayanan, A.L. (2022). Assessment of total aflatoxin (AFB1, AFB2, AFG1 and AFG2) in fish feed and feedstuffs by using high performance thin layer chromatography. *The Pharma Innovation Journal.* **11(9):** 1371-1377. https://doi.org/10.22271/tpi.2022.v11.i9Sq.15535.

Korkut Yıldırım Ali Kop Aysun. and Gurkan Sule. (2018). Length-weight relationship and condition factor as an indicator of growth and feeding intensity of Sea bream *(Sparus aurata* L, 1758) given feed with different protein contents. *Indian Journal of Animal Research*. **53(4):** 510-514. doi: 10. 18805/ijar.B-998.

Kumar, R., Gokulakrishnan, M., Debbarma, J. and Damle, D.K. (2022). Advances in captive breeding and seed rearing of striped murrel *Channa striata*, a high value food fish of Asia. *Animal Reproduction Science.* **238.** https://doi.org/10.1016/j.anireprosci.2022.106957.

Kumari, R., Gupta, S., Singh, A.R., Ferosekhan, S., Kothari, D.C., Pal, A.K. and Jadhao, S.B. (2013). Chitosan nanoencapsulated exogenous trypsin biomimics zymogen-like enzyme in fish gastrointestinal tract. *PloS One*. **8(9).** https://doi.org/10.1371/journal.pone.0074743.

Li, C., Zhang, B., Liu, C., Zhou, H., Wang, X., Mai, K. and He, G. (2020). Effects of dietary raw or *Enterococcus faecium* fermented soybean meal on growth, antioxidant status, intestinal microbiota, morphology and inflammatory responses in turbot (*Scophthalmus maximus* L.). *Fish and Shellfish Immunology*. **100:** 261-271.

Liu, Y., Ding, X., Brown, P.B., Bai, Y., Liu, Z., Shen, J., Liu, H. and Huang, Y. (2023). The digestive enzyme activity, intestinal microbiota and immune-related genes expression of snakehead fish (*Channa argus*) juveniles affected by dietary cricket (*Gryllus testaceus*) meal. *Animal Feed Science and Technology.* **304.**

Love, R.M. (1980). The chemical biology of fishes academic press (11th ed., 467). Academic Press.

Madhubabu, E.P., Jannathulla, R., Imran Khan, H., Ambasankar, K. and Syama Dayal, J. (2021). A blend of plant proteins as a potential fishmeal substitute in the diet of Asian seabass *Lates calcarifer* (Bloch, 1790): Effect on growth, digestive enzymes and fatty acid composition. *Indian Journal of Fisheries*. **68(4):** 65-75. doi: 10.21077/ijf.2021.68.4.108000-08.

Maksimenko, A., Belyi, L., Podvolotskaya, A., Son, O. and Tekutyeva, L. (2024). Exploring sustainable aquafeed alternatives with a specific focus on the ensilaging technology of fish waste. Fermentation. **10(5):** 258. https://doi.org/10.3390/fermentation10050258.

Marklund, S. and Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*. **47(3):** 469-474.

Nazir, M.A., Anjum, K.M., Naseer, J., Anjum, A., Durrani, A.Z., Usman, S., Munir, M.A., Naseer, O. and Usman, M. (2020). Effects of dietary fish oil replacement by soybean meal on performance and physiology of rainbow trout, Oncorhynchus mykiss. *Pakistan Journal of Zoology.* 1-7

NRC. (2011). Nutrient requirements of fish and shrimp. National Academies Press, Washington D.C., USA. https://doi.org/10.17226/13039

Priyatharshni A., Ruby P., Antony Cheryl., Rajagopalsamy C.B.T. (2023). Effect of replacement of soybean meal with sesame meal in the diet of thai-chitralada strain of *Oreochromis niloticus* (L). *Indian Journal of Animal Research*. doi: 10.18805/IJAR.B-5023.

Saliu, F., Leoni, B. and Pergola, R.D. (2017). Lipid classes and fatty acids composition of the roe of wild Silurus glanis from subalpine freshwater. *Food Chemistry*. **232:** 163-168. https://doi.org/10.1016/j.foodchem.2017.04.009.

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. Aquaculture Reports, 30.

Vo, B.V., Bui, D.P., Nguyen, H.Q. and Fotedar, R. (2015). Optimized fermented lupin (*Lupinus angustifolius*) inclusion in juvenile barramundi (*Lates calcarifer*) diets. *Aquaculture*. **444:** 62-69. https://doi. org/10.1016/j.aquaculture.2015.03.019.0.

Wang, J., Tao, Q., Wang, Z., Mai, K., Xu, W., Zhang, Y. and Ai, Q. (2017). Effects of fish meal replacement by soybean meal with supplementation of functional compound additives on intestinal morphology and microbiome of Japanese seabass (*Lateolabrax japonicus*). *Aquaculture Research.* **48(5):** 2186-2197.

Wang, Q.Y., Zhang, G., Zhao, J.B., Zhou, X.J., Dong, W.X., Liu, L. and Zhang, S. (2019). Energy and nutrient digestibility of degossypolized cottonseed protein and its utilization as a protein source in nursery pigs. *Livestock Science.* **223:** 53-59.

Watanabe, T. and Pongmaneerat, J. (1993). Potential of soybean meal as a protein source in extruded pellets for rainbow trout. Nippon Suisan Gakkaishi. **59(8):** 1415-1423.

Xie, K., Liu, X., Shi, Y., Cai, M., Dai, J., Zhang, J. and Hu, Y. (2025). Effects of cholesterol supplementation in high soybean meal diet on Growth, Lipid Metabolism and Intestinal Health of Juvenile Rice Field Eel *Monopterus albus*. *Aquaculture Nutrition*. 2233612. doi: 10.1155/anu/2233612.

Zhang, C., Rahimnejad, S., Wang, Y., Lu, K., Song, K., Wang, L. and Mai, K. (2018). Substituting fish meal with soybean meal in diets for Japanese seabass (Lateolabrax japonicus): Effects on growth, digestive enzymes activity, gut histology and expression of gut inflammatory and transporter genes. *Aquaculture.* **483:** 173-182. doi:10.1016/j.aquaculture.2017.10.

Zhang, Y.Q., Wu, Y.B., Jiang, D.L., Qin, J.G. and Wang, Y. (2014). Gamma-irradiated soybean meal replaced more fish meal in the diets of Japanese. seabass (*Lateolabrax japonicus*). *Animal Feed Science and Technology.* **197:** 155-163. https://doi.org/10.1016/j.anifeedsci.2014.08.002.