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Research Article

Phytase supplementation enhances mineral digestibility and blood indices in *Catla catla* fed plant-based diets

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Keywords

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Abstract

In the fish feed industry, fishmeal (FM) is highly valuable for its excellent combination of essential amino acids and fatty acids. However, due to its high cost and low availability, researchers are focusing on alternative sustainable plant-based protein sources. However, phytate, a major phosphorus-rich compound in plants, has a chelating effect that significantly reduces the absorption and bioavailability of essential nutrients, leading to nutritional imbalances. Adding phytase enzyme (PHY) in aqua feed improves the digestibility of minerals and reduces their excretion. Therefore, this study assessed the impact of PHY supplementation on hematological indices and mineral digestibility in *Catla catla* when fed different plant protein-based diets for FM replacement such as *Moringa oleifera* leaf meal (MOLM), sunflower meal (SFM), canola meal (CM), guar meal (GM). Nine test diets were formulated: a control diet without PHY, and eight experimental diets with PHY added at 250 or 500 FTU/kg. The results revealed that the addition of 500 FTU/kg of PHY to a 50% MOLM significantly enhances the digestibility of minerals like P, Mg, Al, Na, K, Cu, and Zn, when compared to control and other test levels. In terms of blood indices, this supplementation increased the values of RBCs ($3.18\times10^6 mm^{-3}$), Hb (8.68 g/100 ml), PCV (30.07 %) and WBCs ($8.20\times10^3 mm^{-3}$) count as well as serum liver enzymes alanine aminotransferase (0.93 U/L) and aspartate aminotransferase (3.11 U/L). The results indicated that the most effective combination was a diet (T_3) containing 50% MOLM and 500 FTU kg^{-1} PHY, which yielded optimal hematological and mineral profiles. Conclusively, this study shows that PHY-supplemented plant-based fish diets reduce the need for mineral supplements, lowering feed costs and environmental impact.

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Introduction

Indian major carps (IMCs; *Catla catla, Cirrhinus mrigala*, and *Labeo rohita*) dominate the freshwater aquaculture sector, making up around 87% of total production (FAO, 2017; Ghosh *et al*., 2021). They are extensively farmed in India and the surrounding countries. They are native to South Asia and belong to the Cyprinidae family. Due to their high demand, these species hold significant economic value (Hossain *et al*., 2022). *C. catla* is a surface feeder, commonly known as Thaila, and is farmed alongside other fish species in a polyculture system (Aslam *et al.,* 2016). As the sole member of the monotypic genus Catla, it is widely recognized as a fast-growing fish species in southern Asia. As a eurythermal species, *C. catla* grows best in water temperatures between 25 and 32°C (Shakir *et al.,* 2013). Notably, the global *C. catla* output increased by 2.8 million tons in the first decade of the twenty-first century, with continued growth reported in 2012 (FAO, 2015).

In Pakistan, inland and coastal water resources play a vital role in the nutrition and economic growth of cultivable fish species. There is a growing need in the global aquaculture industry to maximize production efficiency while reducing environmental impact. As aquaculture has become more intensive, several challenges have arisen, including the production of affordable, high-quality fish feed and techniques for removing anti-nutrients from feed (Sardar *et al.,* 2007). Aquaculture has emerged as the fastest-growing sector in animal protein production, accounting for almost half of the world's total fish consumption (FAO, 2010, 2022). Feed costs account for approximately 60% of the overall expense of fish farming (Shahzad *et al.,* 2018). Fishmeal (FM) is considered one of the best protein sources for aqua feeds due to its well-balanced amino acid profile (Luthada-Raswiswi *et al.,* 2021). However, FM is relatively expensive and poses sustainability concerns due to overfishing and limited sources (Siddiqui *et al.,* 2022). To address these issues, researchers have explored alternative protein sources, including plant proteins (PPs), which offer a cost-effective and sustainable solution (Chakraborty *et al.,* 2019). Agricultural by-products such as rapeseed meal (Luo *et al.,* 2012), soybean meal (Ye *et al.,* 2011), sunflower meal (SFM) (Iqbal *et al.,* 2022), and cottonseed meal (Hassan *et al.,* 2019) have been identified as potential substitutes for FM.

To achieve sustainable and profitable commercial production, scientists are now exploring unconventional plant-based sources, including seeds, leaves, and other nutrient-rich alternative by-products (Abo-State *et al.,* 2014). *Moringa oleifera*, a member of the Moringaceae family, has emerged as a viable source of protein for fish feed. This fast-growing plant has numerous commercial applications, including food additives and medicinal uses. Moringa leaves have a crude protein content ranging from 25% to 32%, making them a valuable ingredient for sustainable aqua feed production (Shahzad *et al.,* 2017). Moreover, SFM is an affordable and valuable alternative source of essential nutrients, particularly in animal feed and aquaculture. Following soybean meal, cottonseed meal, and rapeseed meal, SFM meal is considered the world's fourth-

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largest protein source (Anjum *et al*., 2014). Canola meal (CM) is another excellent protein source for animal feed. Research has explored the nutritional benefits of CM for various animal species (Enami and Safafar, 2010). CM is used as a feed supplement for numerous fish species, including sea bass, perch, bream, trout, salmon, catfish, carp, and turbot (Enami, 2011). Legume seeds are a valuable ingredient in fish feed due to their high content of protein, carbohydrates, and lipids. One promising leguminous plant is guar (*Cyamopsis tetragonoloba*), which belongs to the Fabaceae family. Guar meal (GM) is commonly used as a nutritious feed supplement for dairy animals in countries like India and Pakistan, and its potential as a fish feed ingredient is worth exploring (Barlaya *et al*., 2021).

The utilization of plant by-products in fish feed is limited due to their high level of indigestible carbohydrates and the existence of anti-nutritional components such as phytate (Laining *et al.,* 2011). Phytate can hinder mineral uptake in fish, but supplementing their diet with phytase (PHY), an enzyme that breaks down phytate, alleviates this issue. The best way to lessen the effects of phytate is to use the enzyme PHY incorporated into the fish diet in various forms, such as microbial PHY (Hussain *et al.,* 2016a,b). By releasing phosphorus and minerals from phytate, microbial PHY reduces inositol phosphate levels. Since agastric and monogastric animals, including fish, lack the bacteria that produce PHY, adding exogenous microbial PHY to plant-based fish diets enhances mineral digestibility and bioavailability. This reduces nutrient loss, especially phosphorus, through fish feces, thereby minimizing aquatic pollution (Priya *et al.,* 2023). PHY deficiency can lead to three main issues: (1) water pollution due to nutrient release from fish, (2) the need for dietary phosphorus supplements, and (3) depletion of phosphorus deposits. Supplementing fish feed with PHY can address nutritional issues by improving phosphorus bioavailability (Hussain *et al.,* 2016a,b). However, this study evaluated the effects of PHY supplementation on hematological profiles and mineral digestibility in *C. catla* fingerlings fed plant-based diets (MOLM, SFM, CM, and GM), aiming to minimize mineral supplement requirements, reduce feed costs, and mitigate environmental impacts.

Materials and methods

Fish and experimental conditions

C. catla (average weight: 6.10±0.02 g) were obtained from the Rahim Yar Khan Fish seed hatchery and were taken to the Fish Nutrition lab, where they were kept in specially designed glass aquaria to collect fish waste. For two weeks, they were remained in these aquaria to acclimatize to the experimental environment. The fingerlings were fed the basal diet twice a day until satiation during the acclimatization phase (Allan and Rowland, 1992). Water quality parameters were monitored constantly using a pH meter (Jenway 3510), a dissolved oxygen (DO) meter (Jenway 970), and an electrical conductivity meter (HANNA: HI. 8633) throughout the trial. A capillary system was used to continuously ventilate each aquarium to keep the water fresh and well-oxygenated.

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Experimental diets and their formulation

Before test diet preparation, feed ingredients were purchased from a commercial feed mill and ground and sieved to a 2 mm particle size (Table 1). Standard procedures were used to examine the chemical composition of test diets and feed ingredients (AOAC, 1995). After mixing the dry components in an electric mixer for ten to twenty minutes, fish oil was added gradually while the mixture was stirred continuously. To examine the mineral digestibility, chromium oxide (1%) was used as an inert marker. Water, constituting approximately 10–15% of the total amount, was added to prepare the dough for each test diet. The dough was then processed via a lab extruder to form floating pellets (Lovell, 1989; Faisal *et al*., 2024). Four plant-based protein sources (MOLM, SFM, CM, and GM) were used as a substitution (15% and 50%) of FM in the test diets. PHY was added to these diets at levels of 250 and 500 FTU/kg, with the exception of the control diet. Nine test diets were formulated, including a control diet (without PHY) and eight experimental diets. Eight test diets (T2-T9) were formulated by spraying varying PHY levels, including 250 and 500 FTU/kg. Plant by-products with a 15% replacement level were supplemented with 250 FTU/kg, while plant meals with a 50% substitution level received 500 FTU/kg supplementation. This study consisted of a 70-day feeding experiment.

Table 1: Ingredients composition (%) of diets.

Ingredients	Test Diet (TD; control)	*Moringa oleifera* leaf meal		Sunflower meal		Canola meal		Guar meal	
	TD-1	TD-2	TD-3	TD- 4	TD-5	TD-6	TD-7	TD-8	TD-9
Plant by-products	-	35	50	35	50	35	50	35	50
Fishmeal	50	15	-	15	-	15	-	15	-
Soybean meal	15	15	15	15	15	15	15	15	15
Rice polish	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Wheat flour	17	17	17	17	17	17	17	17	17
Fish oil	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Vitamin Premix	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Ascorbic acid	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral Premix	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Chromic oxide	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Phytase level	0 FTU kg^{-1}	250 FTU kg^{-1}	500 FTU kg^{-1}	250 FTU kg^{-1}	500 FTU kg^{-1}	250 FTU kg^{-1}	500 FTU kg^{-1}	250 FTU kg^{-1}	500 FTU kg^{-1}

Feeding regimen and sampling procedure

C. catla were fed the recommended diets at 4% of their live wet weight. For each test diet, three replicates were allocated and fifteen fish were randomly assigned to each tank (70 litre water capacity). Following the two-hour feeding session, uneaten feed was manually siphoned from each tank. The

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tanks were then thoroughly cleaned, and the water was refreshed with fresh tap water. Fecal samples were collected from each tank using the specialized feces collection tube. To minimize nutrient leaching, the fecal threads were handled carefully to prevent breakage. The collected feces from each treatment were then dried and preserved for subsequent chemical analysis (Hussain *et al*., 2024).

Blood collection and hematological analysis

Tricaine methanesulfonate (MS-222;150 mg/L solution; 15 minutes) and sodium bicarbonate (as buffer) (Wagner *et al.,* 1997) was used to anesthetize the fish for blood sampling. These blood samples were sent for hematological testing. The packed cell volume (PCV) was determined using a 25 mm capillary tube and centrifuged in a hematocrit microcentrifuge following the Wintrobe and Westergreen technique as explained by Blaxhall and Daisley (1973). A hemocytometer with an improved Neubauer counting chamber, as indicated by Blaxhall and Daisley (1973), was used to calculate white blood cells and red blood cell counts. The hemoglobin (Hb) concentration was estimated using the method explained by Wedemeyer and Yastuke (1977). Blood serum samples were analyzed using a veterinary biochemistry analyzer (Beckman Coulter AU680, USA). This analyzer was used to measure the activities of two key liver enzymes: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Ahmed *et al*., 2023). The other indices *i.e*. mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), and mean cell volume (MCV) were calculated as determined by Hussain *et al.* (2024).

Minerals assessment

A conical flask was filled with a 1-g sample to calculate mineral content, and 20 mL of nitric acid (HNO_3) was added. Then, the flask was set on a heated plate. When the liquid started to boil, 10 mL of perchloric acid were added and the mixture was heated until 1 mL mixture remained. Then the flask was removed and distilled water was added to attain the final volume of 50 mL making it crystal clear. Before mineral testing, the solution was filtered through filter paper to remove all solid particles from the digesting solution. The mineral compositions were calculated by using an atomic absorption spectrophotometer (Hitachi, Z-8200, Japan) after appropriate dilution (AOAC, 1995). A flame photometer (Jenway PFP-7, UK) was used to estimate the amount of sodium and potassium. Phosphorus was analyzed calorimetrically by using a UV/VIS 2001 Spectrophotometer at 350 nm.

Apparent digestibility coefficient calculation

Chromium oxide (Cr_2O_3) is an inert marker used to determine digestibility. Its concentration is determined using an acid digestion method, which involves oxidizing ash samples of feed and feces with perchloric acid ($HCLO_4$), followed by Divakaran (2002). The ADCs for diets were measured by using the formula described in NRC (1993) and Hussain *et al.* (2024).

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Statistical analysis

One-way analysis of variance (ANOVA) was applied to the resulting data (Steel *et al.,* 1996). Tukey's Honesty Significant Difference test (HSD) was used to compare the mean differences, and a p-value less than 0.05 was considered significant (Snedecor and Cochran, 1991). For data analysis, the CoStat computer software (Version 6.4, 93940 USA) was used.

Results

Hematological studies

The hematological indices of *C. catla* fed diets with varied levels of FM replacement with four different plant-based protein sources are shown in Table 2. The fish fed on test diet T_3, which was replaced with 50% of MOLM supplemented with 500 FTU kg^{-1} PHY, showed the highest values of PCV (30.07%), MCHC (33.60%), RBCs ($3.18\times10^6 mm^{-3}$), and WBCs ($8.20\times10^3 mm^{-3}$). Conversely, fish fed on test diet T_7 with 50% CM supplemented with 500 FTU kg^{-1} PHY showed the second-highest values of RBCs ($3.11\times10^6 mm^{-3}$), MCHC (30.27%), WBCs ($8.13\times10^3 mm^{-3}$), and PCV (29.77%) than the fish fed with the control diet. The fish fed with the control diet with 0% plant by-products with no supplementation had the lowest values of RBCs ($2.37\times10^6 mm^{-3}$), WBCs ($6.95\times10^3 mm^{-3}$), PCV (19.93%), and MCHC (21.73%). Similarly, fish fed test diet T_5 (50% SFM supplemented with 500 FTU kg^{-1}) had the maximum values of MCH (70.8 pg) and MCV (210.1 fl), while fish fed the control test diet had the lowest values of MCH (24.47 pg) and T8 had the lowest value of MCV (85.12 fl). The best results were observed when the plant-based diets were replaced by up to 50%. PHY supplementation with plant-based diets played a significant role, both individually and jointly. The highest values of RBCs, WBCs, PCV, and MCHC were observed in MOLM-based diets supplemented with 500 FTU kg^{-1} level of PHY enzyme; whereas 50% CM supplemented with PHY (500 FTU kg^{-1} level) also played a potential role.

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Table 2: Blood parameters of *Catla catla* fed PHY supplemented with four different plant-based protein sources.

Test diets	Fishmeal Protein Replacement Levels	Plant by-products	Plant by-products Replacement Levels	PHY (FTU/kg^1)	RBCs ($10^6 mm^{-3}$)	WBCs ($10^3 mm^{-3}$)	Hb (g/100ml)	PCV (%)	Alanine aminotransferase (U/L)	MCHC (%)	Aspartate aminotransferase (U/L)	MCH (pg)	MCV (fl)
T_1	50	MOLM	0	0	2.37 ± 0.18^{i}	6.95 ± 0.05^{i}	4.53 ± 0.58^{i}	19.93 ± 0.58^{i}	0.86 ± 0.02^{d}	21.73 ± 0.02^{i}	2.43 ± 0.09^{g}	24.47 ± 0.01^{a}	93.73 ± 1.53^{c}
T_2	35		15	250	3.06 ± 0.03^{c}	8.13 ± 0.02^{c}	7.46 ± 0.06^{c}	26 ± 0.58^{c}	0.89 ± 0.03^{bc}	29.73 ± 0.02^{d}	2.96 ± 0.12^{c}	27.81 ± 0.48^{e}	99.49 ± 0.02^{e}
T_3	0		50	500	3.18 ± 0.16^{a}	8.20 ± 0.17^{a}	8.68 ± 0.10^{a}	30.07 ± 0.10^{a}	0.93 ± 0.01^{a}	33.60 ± 0.02^{a}	3.11 ± 0.18^{a}	27.06 ± 0.59^{c}	87.06 ± 0.59^{b}
T_4	35	SFM	15	250	2.93 ± 0.04^{f}	7.96 ± 0.03^{f}	6.68 ± 0.19^{f}	22.01 ± 0.02^{f}	0.89 ± 0.02^{bc}	26.44 ± 0.04^{h}	2.85 ± 0.11^{d}	54.30 ± 0.92^{h}	141.1 ± 0.02^{g}
T_5	0		50	500	2.97 ± 0.02^{e}	8.08 ± 0.07^{e}	6.99 ± 0.02^{e}	24.01 ± 1.01^{e}	0.87 ± 0.05^{c}	28.01 ± 0.02^{e}	2.83 ± 0.08^{d}	70.8 ± 1.56^{i}	210.1 ± 0.99^{i}
T_6	35	CM	15	250	3.02 ± 0.01^{d}	8.09 ± 0.02^{d}	7.20 ± 0.26^{d}	25.34 ± 0.56^{d}	0.89 ± 0.08^{bc}	27.01 ± 0.01^{g}	2.94 ± 0.17^{c}	28.0 ± 0.99^{f}	103.18 ± 1.04^{f}
T_7	0		50	500	3.11 ± 0.10^{b}	8.13 ± 0.02^{b}	7.90 ± 0.01^{b}	29.77 ± 0.02^{b}	0.92 ± 0.04^{ab}	30.27 ± 0.78^{c}	3.06 ± 0.15^{b}	27.61 ± 0.50^{d}	97.43 ± 1.00^{d}
T_8	35	GM	15	250	2.86 ± 0.11^{h}	7.81 ± 0.09^{h}	5.97 ± 0.07^{h}	23.34 ± 0.57^{h}	0.90 ± 0.07^{b}	31.31 ± 0.34^{b}	2.67 ± 0.13^{f}	26.6 ± 0.02^{b}	85.12 ± 1.54^{a}
T_9	0		50	500	2.90 ± 0.66^{g}	7.92 ± 0.04^{g}	6.16 ± 0.01^{g}	23.67 ± 1.14^{g}	0.88 ± 0.03^{c}	27.98 ± 0.08^{f}	2.72 ± 0.17^{e}	49.53 ± 0.55^{g}	141.8 ± 11.26^{h}

Means with distinct superscript letters within a column denote substantial variations ($p<0.05$). The results are presented as mean ± SD of three replicates.

Mineral digestibility

Tables 3-13 presente the impacts of PHY on the mineral digestibility of *C. catla*. Supplementing *C. catla* fingerlings with PHY enzyme resulted in improved mineral digestibility. The maximum mineral digestibility values were 63.12% for Ca,70.78 % for P, 60.56% for Na, 70.76% for K, 52.69% for Cr, 68.08% for Mg, 73.14% for Fe,73.18 % for Cu, 59.84% for Al, 67.20% for Mn, and 63.3% for Zn were observed when fish were fed the T_3 test diet, which included 50% MOLM supplemented with 500 FTU kg^{-1} PHY. When the feed mentioned above was given to *C. catla* fingerlings, the lowest amount of minerals was also observed in their feces.

Table 3: Mineral content analysis (%) of Ca in diets, feces and nutrient absorption assessment of *Catla catla* fed on PHY supplemented four plant-based protein sources.

Test diets	Fishmeal Protein Substitution Levels (%)	Plant by-products	Plant by-products Replacement Levels (%)	PHY (FTU/kg^1)	Diets	Feces	Digestibility
T_1	50	MOLM	0	0	3.21±0.02	2.58 ± 0.02^{a}	23.56 ± 4.84^{i}
T_2	35		15	250	3.21±0.03	1.46 ± 0.04^{g}	57.95 ± 3.58^{c}
T_3	0		50	500	3.21±0.02	1.22 ± 0.03^{i}	63.12 ± 1.48^{a}
T_4	35	SFM	15	250	3.16±0.04	1.86 ± 0.03^{d}	49.88 ± 1.42^{f}
T_5	0		50	500	3.15±0.02	1.75 ± 0.04^{e}	50.17 ± 1.57^{e}

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Test diets	Fishmeal Protein Substitution Levels (%)	Plant by-products	Plant by-products Replacement Levels (%)	PHY (FTU/kg¹)	Diets	Feces	Digestibility
T_6	35	CM	15	250	3.11±0.02	1.65±0.03^{f}	51.50±0.82^{d}
T_7	0		50	500	3.12±0.03	1.23±0.05^{h}	58.79±2.05^{b}
T_8	35	GM	15	250	3.16±0.02	2.08±0.02^{b}	40.23±1.05^{h}
T_9	0		50	500	3.16±0.03	1.95±0.02^{c}	45.56±1.56^{g}

Means with distinct superscript letters within a column denote substantial variations (p<0.05). The results are presented as mean ± SD of three replicates.

Table 4: Mineral content analysis (%) of Na in diets, feces and nutrient absorption assessment of *Catla catla* fed on PHY supplemented four plant-based protein sources.

Test diets	Fishmeal Protein Substitution Levels (%)	Plant by-products	Plant by-products Replacement Levels (%)	PHY (FTU/kg¹)	Diets	Feces	Digestibility
T_1	50	MOLM	0	0	0.22±0.02	0.236±0.003^{a}	33.17±1.37^{i}
T_2	35		15	250	0.21±0.03	0.117±0.002^{h}	55.23±0.14^{c}
T_3	0		50	500	0.23±0.03	0.103±0.025^{i}	60.56±1.40^{a}
T_4	35	SFM	15	250	0.24±0.03	0.146±0.003^{d}	48.33±0.49^{f}
T_5	0		50	500	0.25±0.02	0.139±0.007^{e}	50.56±0.44^{e}
T_6	35	CM	15	250	0.27±0.02	0.126±0.003^{g}	52.23±0.14^{d}
T_7	0		50	500	0.26±0.02	0.134±0.045^{f}	58.51±0.30^{b}
T_8	35	GM	15	250	0.24±0.03	0.175±0.001^{b}	44.33±1.60^{h}
T_9	0		50	500	0.24±0.05	0.161±0.005^{c}	46.88±2.32^{g}

Means with distinct superscript letters within a column denote substantial variations (p<0.05). The results are presented as mean ± SD of three replicates.

Table 5: Mineral content analysis (%) of K in diets, feces and nutrient absorption assessment of *Catla catla* fed on PHY supplemented four plant-based protein sources.

Test diets	Fishmeal Protein Substitution Levels (%)	Plant by-products	Plant by-products Replacement Levels (%)	PHY (FTU/kg¹)	Diets	Feces	Digestibility
T_1	50		0	0	0.26±0.02	0.246±0.004^{a}	36.62±0.12^{i}
T_2	35	MOLM	15	250	0.26±0.03	0.136±0.004^{g}	63.09±0.91^{c}
T_3	0		50	500	0.27±0.03	0.086±0.012^{i}	70.76±1.41^{a}
T_4	35	SFM	15	250	0.27±0.03	0.173±0.002^{d}	51.45±0.14^{f}
T_5	0		50	500	0.25±0.03	0.167±0.005^{e}	55.46±0.17^{e}
T_6	35	CM	15	250	0.26±0.01	0.150±0.002^{c}	59.62±0.12^{d}
T_7	0		50	500	0.25±0.02	0.122±0.002^{h}	65.46±0.29^{b}
T_8	35	GM	15	250	0.25±0.02	0.197±0.002^{b}	47.46±0.29^{h}
T_9	0		50	500	0.24±0.02	0.183±0.002^{c}	50.45±0.14^{g}

Means with distinct superscript letters within a column denote substantial variations (p<0.05). The results are presented as mean±SD of three replicates.

Table 6: Mineral content analysis (%) of P in diets, feces and nutrient absorption assessment of *Catla catla* fed on PHY supplemented four plant-based protein sources.

Test diets	Fishmeal Protein Substitution Levels (%)	Plant by-products	Plant by-products Replacement Levels (%)	PHY (FTU/kg¹)	Diets	Feces	Digestibility
T_1	50		0	0	0.515±0.003	0.535±0.002^{a}	34.65±0.81^{i}
T_2	35	MOLM	15	250	0.515±0.004	0.356±0.020^{f}	54.32±1.15^{c}
T_3	0		50	500	0.515±0.002	0.284±0.003^{i}	70.78±1.36^{a}
T_4	35	SFM	15	250	0.516±0.003	0.432±0.011^{d}	44.99±3.94^{f}
T_5	0		50	500	0.513±0.002	0.414±0.003^{e}	45.68±3.66^{e}
T_6	35	CM	15	250	0.514±0.003	0.319±0.007^{g}	48.45±2.14^{d}
T_7	0		50	500	0.514±0.003	0.313±0.002^{h}	59.60±1.00^{b}
T_8	35	GM	15	250	0.515±0.003	0.495±0.004^{b}	40.74±3.47^{h}
T_9	0		50	500	0.514±0.004	0.464±0.003^{c}	43.08±2.43^{g}

Results are presented as mean±SD (N=3). Values with distinct superscripts in the same column indicate significant variations (p<0.05).

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Table 7: Mineral content analysis (%) of Fe in diets, feces and nutrient absorption assessment of *Catla catla* fed on PHY supplemented four plant-based protein sources.

Test diets	Fishmeal Protein Substitution Levels (%)	Plant by-products	Plant by-products Replacement Levels (%)	PHY (FTU/kg^{1})	Diets	Feces	Digestibility
T_1	50	MOLM	0	0	0.019±0.010	0.249±0.013^{a}	33.42±0.09^{i}
T_2	35		15	250	0.014±0.00	0.101±0.002^{g}	52.14±0.93^{c}
T_3	0		50	500	0.013±0.00	0.009±0.002^{i}	73.14±0.96^{a}
T_4	35	SFM	15	250	0.013±0.001	0.153±0.001^{d}	45.37±2.85^{f}
T_5	0		50	500	0.013±0.00	0.140±0.003^{e}	46.85±1.91^{e}
T_6	35	CM	15	250	0.013±0.00	0.131±0.004^{f}	49.52±1.46^{d}
T_7	0		50	500	0.013±0.00	0.009±0.003^{h}	53.86±4.87^{b}
T_8	35	GM	15	250	0.013±0.00	0.172±0.001^{b}	42.70±3.16^{h}
T_9	0		50	500	0.012±0.001	0.161±0.003^{c}	43.37±3.41^{g}

Means with distinct superscript letters within a column denote substantial variations (p<0.05). The results are presented as mean ± SD of three replicates.

Table 8: Mineral content analysis (%) of Cu in diets, feces and nutrient absorption assessment of *Catla catla* fed on PHY supplemented four plant-based protein sources.

Test diets	Fishmeal Protein Substitution Levels (%)	Plant by-products	Plant by-products Replacement Levels (%)	PHY (FTU/kg^{1})	Diets	Feces	Digestibility
T_1	50	MOLM	0	0	0.0113±0.0004	0.01598±0.00002^{a}	33.79±5.76^{i}
T_2	35		15	250	0.0115±0.0006	0.01006±0.00003^{g}	49.85±0.29^{c}
T_3	0		50	500	0.0121±0.0002	0.00766±0.00028^{a}	73.18±3.53^{a}
T_4	35	SFM	15	250	0.0124±0.0005	0.01195±0.00002^{d}	45.38±3.59^{f}
T_5	0		50	500	0.0122±0.0002	0.01116±0.00003^{e}	46.05±5.64^{e}
T_6	35	CM	15	250	0.0122±0.0001	0.01086±0.00003^{f}	47.18±1.71^{d}
T_7	0		50	500	0.0125±0.0001	0.00929±0.00012^{h}	60.39±1.05^{b}
T_8	35	GM	15	250	0.0117±0.0002	0.01298±0.00002^{b}	41.14±0.93^{h}
T_9	0		50	500	0.0118±0.0002	0.01235±0.00002^{c}	43.15±0.92^{g}

Means with distinct superscript letters within a column denote substantial variations (p<0.05). The results are presented as mean ± SD of three replicates.

Table 9: Mineral content analysis (%) of Zn in diets, feces and nutrient absorption assessment of *Catla catla* fed on PHY supplemented four plant-based protein sources.

Test diets	Fishmeal Protein Substitution Levels (%)	Plant by-products	Plant by-products Replacement Levels (%)	PHY (FTU/kg^{1})	Diets	Feces	Digestibility
T_1	50	MOLM	0	0	0.0288±0.0004	0.0185±0.0002^{g}	60.3±1.11^{c}
T_2	35		15	250	0.0291±0.0003	0.0123±0.0002^{i}	63.3±1.82^{a}
T_3	0		50	500	0.0290±0.0001	0.0274±0.0003^{d}	55.47±4.05^{f}
T_4	35	SFM	15	250	0.0293±0.0004	0.0215±0.0004^{e}	58.3±1.94^{e}
T_5	0		50	500	0.0290±0.0004	0.0197±0.0002^{f}	59.3±0.75^{d}
T_6	35	CM	15	250	0.0286±0.0004	0.0154±0.0002^{h}	62.63±1.06^{b}
T_7	0		50	500	0.0292±0.0009	0.0306±0.0003^{b}	51.47±1.19^{h}
T_8	35	GM	15	250	0.0294±0.0004	0.0296±0.0003^{c}	52.75±6.07^{g}
T_9	0		50	500	0.0288±0.0004	0.0185±0.0002^{g}	60.3±1.11^{c}

Means with distinct superscript letters within a column denote substantial variations (p<0.05). The results are presented as mean ± SD of three replicates.

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Table 10: Mineral content analysis (%) of Mn in diets, feces and nutrient absorption assessment of *Catla catla* fed on PHY supplemented four plant-based protein sources.

Test diets	Fishmeal Protein Substitution Levels (%)	Plant by-products	Plant by-products Replacement Levels (%)	PHY (FTU/kg^1)	Diets	Feces	Digestibility
T_1	50	MOLM	0	0	0.0113±0.0001	0.0184±0.0002^a	31.40±0.78^i
T_2	35		15	250	0.0117±0.0001	0.0100±0.0004^g	54.07±5.65^c
T_3	0		50	500	0.0119±0.0002	0.0077±0.0002^i	67.20±1.26^a
T_4	35	SFM	15	250	0.0117±0.0003	0.0130±0.0005^d	48.92±3.26^f
T_5	0		50	500	0.0121±0.0002	0.0120±0.0002^e	50.40±1.33^e
T_6	35	CM	15	250	0.0114±0.0004	0.0111±0.0003^f	52.64±1.05^d
T_7	0		50	500	0.0120±0.0004	0.0099±0.0002^h	56.97±3.10^b
T_8	35	GM	15	250	0.0118±0.0005	0.0154±0.0002^b	44.92±2.82^h
T_9	0		50	500	0.0119±0.0006	0.0149±0.0003^c	47.87±3.97^g

Means with distinct superscript letters within a column denote substantial variations ($p<0.05$). The results are presented as mean ± SD of three replicates.

Table 11: Mineral content analysis (%) of Mg in diets, feces and nutrient absorption assessment of *Catla catla* fed on PHY supplemented four plant-based protein sources.

Test diets	Fishmeal Protein Substitution Levels (%)	Plant by-products	Plant by-products Replacement Levels (%)	PHY (FTU/kg^1)	Diets	Feces	Digestibility
T_1	50	MOLM	0	0	0.0055±0.0004	0.2487±0.012^a	33.70±0.16^i
T_2	35		15	250	0.0054±0.0004	0.1013±0.0003^g	50.23±7.47^c
T_3	0		50	500	0.0051±0.0001	0.0089±0.0002^i	68.08±5.90^a
T_4	35	SFM	15	250	0.0055±0.0004	0.1534±0.0003^d	41.21±0.90^f
T_5	0		50	500	0.0221±0.0291	0.1403±0.0003^e	44.63±0.34^e
T_6	35	CM	15	250	0.0055±0.0003	0.1313±0.0003^f	45.62±3.53^d
T_7	0		50	500	0.0051±0.0001	0.0091±0.0002^h	54.58±0.45^b
T_8	35	GM	15	250	0.0058±0.0002	0.1721±0.0011^b	38.18±4.19^h
T_9	0		50	500	0.0055±0.0002	0.1614±0.0003^c	39.26±3.48^g

Means with distinct superscript letters within a column denote substantial variations ($p<0.05$). The results are presented as mean ± SD of three replicates.

Table 12: Mineral content analysis (%) of Cr in diets, feces and nutrient absorption assessment of *Catla catla* fed on PHY supplemented four plant-based protein sources.

Test diets	Fishmeal Protein Substitution Levels (%)	Plant by-products	Plant by-products Replacement Levels (%)	PHY (FTU/kg^1)	Diets	Feces	Digestibility
T_1	50	MOLM	0	0	0.032±0.004	0.535±0.002^a	25.48±0.37^i
T_2	35		15	250	0.031±0.004	0.356±0.020^g	43.89±3.64^c
T_3	0		50	500	0.031±0.003	0.284±0.003^i	52.69±1.64^a
T_4	35	SFM	15	250	0.033±0.003	0.432±0.011^d	38.81±2.62^f
T_5	0		50	500	0.028±0.008	0.414±0.003^e	40.07±5.17^e
T_6	35	CM	15	250	0.029±0.003	0.391±0.007^f	42.72±0.87^d
T_7	0		50	500	0.025±0.003	0.313±0.002^h	45.55±0.57^b
T_8	35	GM	15	250	0.024±0.003	0.495±0.004^b	35.89±0.96^h
T_9	0		50	500	0.029±0.003	0.464±0.003^c	37.55±0.57^g

Means with distinct superscript letters within a column denote substantial variations ($p<0.05$). The results are presented as mean ± SD of three replicates.

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Table 13: Mineral content analysis (%) of Al in diets, feces and nutrient absorption assessment of *Catla catla* fed on PHY supplemented four plant-based protein sources.

Test diets	Fishmeal Protein Substitution Levels (%)	Plant by-products	Plant by-products Replacement Levels (%)	PHY (FTU/kg[1])	Diets	Feces	Digestibility
T_1	50	MOLM	0	0	0.00018±0.00001	0.01840±0.00019[a]	26.64±5.77[i]
T_2	35		15	250	0.00018±0.00001	0.01004±0.00002[g]	46.51±1.23[c]
T_3	0		50	500	0.00018±0.00001	0.00767±0.00019[i]	59.84±5.50[a]
T_4	35	SFM	15	250	0.00018±0.00001	0.01303±0.00002[d]	38.38±3.85[f]
T_5	0		50	500	0.00018±0.00001	0.01204±0.00002[e]	39.75±5.86[e]
T_6	35	CM	15	250	0.00042±0.00042	0.01114±0.00002[f]	42.51±0.65[d]
T_7	0		50	500	0.00018±0.00001	0.00994±0.00002[h]	48.42±0.99[b]
T_8	35	GM	15	250	0.00018±0.00001	0.01540±0.00019[b]	35.05±2.11[h]
T_9	0		50	500	0.00018±0.00001	0.01493±0.00002[c]	36.64±0.82[g]

Means with distinct superscript letters within a column denote substantial variations ($p<0.05$). The results are presented as mean ± SD of three replicates.

The table below explains how these values differ significantly from the other test diets. These findings revealed that for fish to perform at their best in mineral digestibility, 500 FTU kg^{-1} of PHY supplementation is required. When comparing the digestibility values obtained at diet T_3 to the control diet and the other replacement levels ($p<0.05$). The second highest level, T_7, which released the least amount of minerals through waste, was likewise supplemented with 500 FTU kg^{-1} of PHY along with 50% of CM in place of FM. Second highest mineral digestibility values of Ca (58.79%), P (59.60), Na (58.51%), K (65.46%), Cr (45.55%), Mg (54.58%), Fe (53.86%), Cu (60.39%), Al (48.42%), Mn (56.97%), and Zn (62.63%) were recorded. The lowest mineral digestibility values were observed in T_1 diet. The mineral digestibility of *C. catla* fish was positively affected by PHY. In conclusion, this study showed that MOLM and CM-based diets supplemented with PHY had the potential to improve the mineral digestibility of *C. catla* fingerlings both individually and collectively.

Discussion

This study investigated the impact of PHY supplementation on the hematological and mineral digestibility profiles of *C. catla* fingerlings fed plant-based diets (MOLM, SFM, CM, and GM), with the goal of reducing the need for mineral supplements, lowering production costs, and minimizing environmental impact.

Hematological indices are valuable markers for assessing fish stress, nutritional status, and overall health (Khalafalla *et al.,* 2020), and also reflect physiological alterations in fish (Ranjan *et al*., 2021). Moreover, these are crucial for assessing the quality of feed and its impact on fish health (Shahzad *et al*., 2017). Analysis of ALT and AST levels in blood serum provides valuable insights into the liver health of fish, enabling researchers to detect potential liver damage or disease (Ahmed *et al*., 2023). The current study revealed that fish fed with diet T_3, which

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replaced 50% of MOLM with 500 FTU kg^{-1} PHY, exhibited the highest values for PCV (30.07 %), MCHC (33.60 %), RBCs (3.18×10^6mm^{-3}), ALT (0.93 U/L) and AST (3.11 U/L) and WBCs (8.20×10^3mm^{-3}). Notably, 50 % CM supplemented with PHY (500 FTU kg^{-1}) also showed significant benefits. Overall, supplementing plant-based meal diets with PHY had a substantial impact, both individually and in combination. Nasr *et al*. (2021) suggested that replacing plant by-products such as SFM and soybean meal has beneficial outcomes in response to hemato-biochemical parameters. Notably, replacing fishmeal with plant-based protein sources stimulated an increase in goblet cells, which secrete mucus, and thickened the muscularis layer. This enhancement is likely due to boost mucus secretion and expand absorptive surfaces, ultimately supporting improved gut health and nutrient uptake (Nasr *et al*., 2021).

Supporting findings showed that Atlantic cod (*Gadus morhua*) found that PHY supplementation increased WBC count compared to a control diet (Lazado *et al*., 2010). Another study reported a slight increase in Hb levels in fish fed a PHY-supplemented diet, although the difference was not statistically significant (Yoo and Bai, 2014). PHY supplementation at 500 FTU kg^{-1} has also been shown to improve hematocrit and Hb levels in fish (Sardar *et al.,* 2007). Furthermore, a study on *Orechromis niloticus* found that a soybean meal-based diet supplemented with PHY at 500 FTU kg^{-1} resulted in better hematological responses, including higher RBC and WBC counts, compared to a Jatropha meal-based diet (Kumar *et al*., 2010). Existing research on the impact of PHY on fish hematology is scarce, highlighting a significant knowledge gap in this area.

Phytate, a storage form of phosphorus in plants, strongly binds to minerals, making them less available for digestion and absorption in fish (Nwanna *et al.,* 2007; Soetan and Oyewole, 2009). The release and absorption of vital minerals are improved when the PHY enzyme action breaks down the chelated structure of phytate. Many studies have suggested that phytate binds minerals, such as Mg Mn, Ni, Cr, Fe, P, and K, forming insoluble complexes that are less bioavailable (Hussain *et al.,* 2016a,b). This study found that adding PHY to diets based on MOLM and CM enhanced mineral absorption in *C. catla* fingerlings by breaking down phytate-mineral complexes. The maximum mineral digestibility values for P- 70.78%, Ca- 63.12%, Na- 60.56%, Cr- 52.69%, Mg- 68.08%, Cu- 73.18%, K- 70.76%, Fe- 73.14%, Al- 59.84%, Mn- 67.20%, and Zn- 63.3% were observed when fingerlings were fed T_3 diet, 50% MOLM supplemented with 500 FTU kg^{-1} of PHY. Similarly, when *C. catla* were fed the same T_3 diet, their feces also showed the lowest mineral content. In conclusion, this study demonstrates that supplementing MOLM and CM diets with PHY can improve mineral digestibility in *C. catla* fingerlings, individually and collectively.

Shahzad *et al*. (2018) found that PHY inclusion enhanced mineral absorption in *C. catla* fed MOSM-based diets. Similarly, Cheng and Hardy (2002) reported increased mineral utilization when PHY was added to

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plant meal-based diets, as it released minerals bound to phytate in plant feed ingredients. Zhu *et al*. (2014) also noted a significant reduction in fecal mineral content after PHY supplementation. Furthermore, Liu *et al*. (2013) noted a substantial increase in Ca and P absorption in Grass carp fed PHY-supplemented diets. The optimal level of PHY supplementation depends on the type of plant ingredients in the diet and the specific fish species being researched (Baruah *et al*., 2007). According to Marjan *et al*. (2014) stated that PHY inclusion enhances mineral absorption. Specifically, the optimal PHY level for achieving maximum mineral absorption (ADC%) in *Labeo rohita* was found to be 750 FTU kg^{-1}. This level resulted in significantly higher absorption of essential minerals (Fe, Zn, Na, Ca, Cu, Mn, P, and K) when compared to a control diet, particularly when a dry bread meal-based diet was used as a supplement.

In line with our research, Hussain *et al*. (2016a) recorded maximum mineral utilization in *L. rohita* fed a diet consisting of corn soybean meal (CSM), 400 FTU kg^{-1} PHY, and 4% citric acid. However, it's essential to note that various factors can significantly impact the effectiveness of PHY supplementation, including phytate levels, digestive system pH, fish species, experimental conditions, feed processing, PHY quality, and feed preparation methods (Dersjant-Li *et al*., 2015). The present study's outcomes suggest that increased mineral uptake and utilization, facilitated by PHY supplementation, can lead to reduced water pollution and improved health in *C. catla* fingerlings.

Conclusion

The study revealed that PHY supplementation with plant by-products significantly improved the hematological and mineral profiles of *C. catla*. It was noted that the highest hematological parameters and mineral digestibility rates were achieved with the T_3 diet, which consisted of 50% MOLM and 500 FTU kg^{-1} of PHY. Optimal results were achieved with a diet consisting of 50% MOLM and 500 FTU kg^{-1} PHY, in contrast with other diets and control. In conclusion, the present study reveals that PHY-supplemented plant-based fish diets can decrease the need for additional mineral supplements, resulting in lower feed costs and reduced environmental impact through minimized waste discharge into aquatic ecosystems. Future research should prioritize the development of more economical and eco-friendly aquafeed solutions.

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Conflicts of interest

The Authors declare that there is no conflict of interest.

References

Abo-State, H., Hammouda, Y., El-Nadi, A. and AboZaid, H., 2014. Evaluation of feeding raw moringa (*Moringa oleifera* Lam.) leaves meal in Nile tilapia fingerlings (*Oreochromis niloticus*) diets. *Global Veterineria,* 13(**1**), 10-111.

[Downloaded from jifro.ir on 2026-02-05]

DOI: 10.5829/idosi.gv.2014.13.01.84228

Ahmed, I., Khan, Y.M., Lateef, A., Jan, K., Majeed, A., and Shah, M.A., 2023. Effect of fish meal replacement by Azolla meal on growth performance, hemato-biochemical and serum parameters in the diet of scale carp, *Cyprinus carpio* var. communis. *Journal of the World Aquaculture Society*, 54(**5**), 1301-1316. DOI: 10.1111/jwas.13045

Allan, G.L. and Rowland, S.J., 1992. Development of an experimental diet for silver perch (*Bidynus bidyanus*). *Austasia Aquaculture*, 6, 39-40.

Anjum, M.A., Hussain, Z., Khan, S.H., Ahmad, N., Amer, M.Y. Iftikhar, N., 2014. Assessment of poultry feed ingredients used in commercial compound feed. *Pakistan Journal of Life and Social Sciences,* 12(**2**), 69-73.

AOAC (Association of Official Analytical Chemists)., 1995. Official methods of analysis (15th ed.). Association of Official Analytical chemists, Washington D.C., USA. 1094 P.

Aslam, S., Abbas, S., Kalhoro, M. A. and Shoaib, A., 2016. Anchor worms (lernaeid parasites), *Lernaea polymorpha* yü and *Lernaea cyprinacea* (copépode: lernaeidae) on major carps at different fish farms in Punjab, Pakistan. *Science International*, 28(**1**), 295-298.

Barlaya, G., Ananda Kumar, B.S., Huchchappa, R.C., Basumatary, P., and Kannur, H., 2021. Effect of fish meal replacement with toasted guar meal on growth, food conversion, digestive enzyme activity and final carcass composition of rohu, *Labeo rohita*. *Aquaculture Research*, 52(**11**), 5551-5557. DOI: 10.1111/are.15361

Baruah, K., Sahu, P.N., Pal, K.A., Jain, K.K., Debnath, D. and Mukherjee, C.S., 2007. Dietary microbial phytase and citric acid synergistically enhances nutrient digestibility and growth performance of *Labeo rohita* (Hamilton) juveniles at sub-optimal protein level. *Aquaculture Research*, *38,* 109-120. DOI:10.1111/j.1365-2109.2006.01607.x

Blaxhall, P.C. and Daisley, K.W., 1973. Routine haematological methods for use with fish blood. *Journal of Fish Biology,* 6, 771-781. DOI:10.1111/j.1095-8649.1973.tb04510.x

Chakraborty, P., Mallik, A., Sarang, N. and Lingam, S.S., 2019. A review on alternative plant protein sources available for future sustainable aqua feed production. *International Journal of Chemical Studies*, 7(**3**), 1399-1404.

Cheng, Z.J. and Hardy, R.W., 2002. Effect of microbial phytase on apparent nutrient digestibility of barley, canola meal, wheat and wheat middlings, measured in vivo using rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition*, 8, 271-277. DOI:10.1046/j.1365-2095.2002.00152.x

Dersjant-Li, Y., Awati, A., Schulze, H. and Partridge, G., 2015. Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. *Journal of the Science of Food and Agriculture*, 95(**5**), 878-896. DOI:10.1002/jsfa.6998

[Downloaded from jifro.ir on 2026-02-05]

Divakaran, S., Leonard, G.O., and Lan, P.F., 2002. Note on the methods for determination of chromic oxide in shrimp feeds. *Journal of Agriculture and Food Chemistry*, 50, 464-467. DOI:10.1021/jf011112s

Enami, H.R. and Safafar H., 2010. Evaluation of adding canola meal to diet on growth performance of male wistar rats. *Asian Journal of Animal and Veterinary Advances*, 5, 478-483.

Enami, H.R., 2011. A review of using canola/rapseed meal in aquaculture feeding. *Journal of Fisheries and Aquatic Science,* 1, 22-36. DOI:10.3923/jfas.2011.22.36

Faisal, M., Hussain, S.M., Hussain, M., Yousaf, Z., and Amjad, M., 2024. Potential of *Moringa oleifera* leaf meal as substitute of fishmeal on performance of *Catla catla* fingerlings reared in earthen ponds. *Pakistan Journal of Zoology*, 56(4). DOI: DOI:10.17582/journal.pjz/20221202061259

FAO., 2010. Fisheries and aquaculture technical paper. Food and Agriculture Organization of the United Nations, Rome. 70 P.

FAO., 2015. Fisheries and Aquaculture Department, *Catla catla* (Hamilton, 1822) Cultured Aquatic Species Information Programme. Food and Agriculture Organization of the United Nations.

FAO., 2017. Building climate-resilient fisheries and aquaculture in the Asia-pacific region. FAO/APFIC Regional Consultative Workshop. Bangkok, Thailand, 14-16 November 2017.

FAO., 2022. The state of world fisheries and aquaculture- towards blue transformation. Food and Agriculture Organization of the United Nations, Rome. 266 P. DOI: 10.4060/cc0461en

Ghosh, K., Mukherjee, A., Dutta, D., Banerjee, S., Breines, E. M., Hareide, E., and Ringø, E., 2021. Endosymbiotic pathogen-inhibitory gut bacteria in three Indian major carps under polyculture system: A step toward making a probiotics consortium. *Aquaculture and Fisheries*, 6(2), 192-204. DOI:10.1016/j.aaf.2020.07.005

Hussain, S.M., Afzal, M., Nasir, S., Javid, A., Azmat, H., Mamoona Makhdoom, S., Shah, S.H.Z., Hussain, M., Mustafa, I. and Iqbal, M., 2016a. Role of phytase supplementation in improving nutrient digestibility and growth performance for *Labeo rohita* fingerlings fed on canola meal-based diet. *Journal of Applied Animal Research*, 45(**1**), 15-21. DOI:10.1080/09712119.2015.1021814

Hussain, S.M., Ahmad, S., Shahzad, M.M., Arsalan, M.Z.H., Riaz, D., Ahmad, N., and Ahmed, A.W., 2016b. Mineral digestibility of *Labeo rohita* fingerlings fed on cottonseed meal based diets supplemented with citric acid and phytase enzyme. *International Journal of Biosciences*, 8(**2**), 25-35. DOI:10.12692/ijb/8.2.25-35

Hassaan, M.S., El-Sayed, A.I.M., Soltan, M.A., Iraqi, M.M., Goda, A.M., Davies, S.J., and Ramadan, H.A., 2019. Partial dietary fish meal replacement with cotton seed meal and supplementation with exogenous protease alters growth, feed

[Downloaded from jifro.ir on 2026-02-05]

performance, hematological indices and associated gene expression markers (GH, IGF-I) for Nile tilapia, *Oreochromis niloticus*. *Aquaculture*, 503, 282-292. DOI:10.1016/j.aquaculture.2018.12.042

Hossain, M.B., Nur, A.A.U., Ahmed, M.M., Ullah, M.A., Albeshr, M.F., and Arai, T., 2022. Growth, yield and profitability of Major Carps culture in coastal homestead ponds stocked with wild and hatchery fish seed. *Agriculture*, 12(8), 1131-1138. DOI:10.3390/agriculture12081131

Hussain, S. M., Naeem, E., Ali, S., Adrees, M., Riaz, D., Paray, B.A., and Naeem, A. 2024. Evaluation of growth, nutrient absorption, body composition and blood indices under dietary exposure of iron oxide nanoparticles in Common carp (*Cyprinus carpio*). *Journal of Animal Physiology and Animal Nutrition*, 108(2), 366-373. DOI:10.1111/jpn.13892

Iqbal, M., Yaqub, A. and Ayub, M., 2022. Effects of partial and full dietary substitution of fish meal and soybean meal by sunflower meal on growth performance, feed consumption, body indices, serum chemistry and intestine morphology of *Oreochromis niloticus. Turkish Journal of Fisheries and Aquatic Sciences*, 22(**10**). DOI:10.4194/1303-2712-v22_10_05

Khalafalla, M.M., Ibrahim, S.A., Zayed, M.M., Awad, M.N. and Mohamed, R.A., 2020. Effect of a dietary mixture of beneficial bacteria on growth performance, health condition, chemical composition, and water quality of Nile Tilapia, *Oreochromis niloticus* fingerlings. *Journal of Aquatic Food Product Technology*, 29(**8**), 823-835. DOI:10.1080/10498850.2020.1791943

Kumar, V., Makkar, H.P., Amselgruber, W. and Becker, K., 2010. Physiological, haematological and histopathological responses in common carp (*Cyprinus carpio* L.) fingerlings fed with differently detoxified *Jatropha curcas* kernel meal. *Food and Chemical Toxicology*, 48(**8-9**), 2063-2072. DOI:10.1016/j.fct.2010.05.006

Laining A., Ishikawa M., Kyaw K., Gao J., Binh N.T., Koshio S., Yamaguchi S., Yokoyama, S. and Koyama, J., 2011. Dietary Calcium and phosphorus ratio influences the efficiency of microbial phytase on growth, mineral digestibility and vertebral mineralization in juvenile tiger puffer. *Aquaculture Nutrition,* 17, 267-277. DOI:10.1111/j.1365-2095.2010.00768.x

Lazado, C.C., Caipang, C.M.A., Gallage, S., Brinchmann, M.F. and Kiron, V., 2010. Responses of Atlantic cod *Gadus morhua* head kidney leukocytes to phytase produced by gastrointestinal-derived bacteria. *Fish Physiology and Biochemistry*, 36(**4**), 883-891. DOI:10.1007/s10695-010-9375-3

Liu, L.W., Su, J.M., Zhang, T., Liang, X.F. and Luo, Y.L., 2013. Apparent digestibility of nutrients in grass carp (*Ctenopharyngodon idellus*) diet supplemented with graded levels of neutral phytase using pretreatment and spraying methods. *Aquaculture Nutrition*, 19(**1**), 91-99. DOI:10.1111/j.1365-2095.2012.00941.x

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Lovell, T., 1989. Nutrition and feeding of fish. Van Nostrand Reinhold, New York.

Luo, Z., Liu, C.X. and Wen, H., 2012. Effect of dietary fish meal replacement by canola meal on growth performance and hepatic intermediary metabolism of genetically improved farmed tilapia strain of Nile tilapia, *Oreochromis niloticus,* reared in fresh water. *Journal of the World Aquaculture Society*, 43(**5**), 670-678. DOI:10.1111/j.1749-7345.2012.00563.x

Luthada-Raswiswi, R., Mukaratirwa, S. and O'brien, G., 2021. Animal protein sources as a substitute for fishmeal in aquaculture diets: A systematic review and meta-analysis. *Applied Sciences*, 11(**9**), 3854. DOI:10.3390/app11093854

Marjan, S., Afzal, M., Shah, S.Z.H., Hussain, S. M. and Mubarik, M.S., 2014. Role of phytase supplementation in improving mineral digestibility of dry bread meal based diet fed to *Labeo rohita* fingerlings. *Pakistan Journal of Life and Social Sciences*, 12, 150-154.

Nasr, M.A., Reda, R.M., Ismail, T.A., and Moustafa, A., 2021. Growth, hemato-biochemical parameters, body composition, and myostatin gene expression of *Clarias gariepinus* fed by replacing fishmeal with plant protein. *Animals*, 11(3), 889. DOI:10.3390/ani11030889

National Research Council (NRC), 1993. Nutrient requirements of fish. National Academy Press. Washington.

Nwanna, L.C., Eisenreich, R. and Schwarz, F.J., 2007. Effect of wet-incubation of dietary plant feedstuffs with phytases on growth and mineral digestibility by common carp *Cyprinus carpio* L. *Aquaculture*, 271(**1**), 461-468. DOI:10.1016/j.aquaculture.2007.06.010

Priya, Virmani, I., Pragya, Goswami, R.K., Singh, B., Sharma, J.G. and Giri, B., 2023. Role of microbial phytases in improving fish health. *Reviews in Aquaculture*, 15(**4**), 1480-1500. DOI:10.1111/raq.12768

Ranjan, A., Kumar, S., Sahu, N.P., Jain, K.K. and Deo, A.D., 2021. Exogenous phytase and xylanase supplementation of formulated diets for rohu (*Labeo rohita*): Impact on haematology, histology and IGF I gene expression. *Fish Physiology and Biochemistry*, 47, 49-58. DOI:10.1007/s10695-020-00818-8

Sardar, P., Randhawa, H.S., Abid, M. and Prabhakar, S.K., 2007. Effect of dietry microbial phytase supplementation on growth performance, nutrient utilization, body compositions and haemato-biochemical profiles of *Cyprinus carpio* (L.) fingerlings fed soyprotein- based diet. *Aquaculture Nutrition,* 13, 444-456. DOI:10.1111/j.1365-2095.2007.00486.x

Shahzad, M.M., Hussain, S.M., Jabeen, F., Hussain, A.I., Ahmad, S., Ashraf, A. and Arsalan, M.Z.U.H., 2017. Effect of phytase supplementation on mineral digestibility to *Catla catla* fingerlings fed *Moringa oleifera* leaf meal-based test diets. *Punjab University Journal of Zoology*, 32(**1**), 65-73.

Shahzad, M.M., Hussain, S.M., Jabeen, F., Hussain, A.I., Javid, A., Asrar, M. and Arsalan, M.Z.U.H., 2018. Improvement in Mineral Digestibility

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and Whole Body Composition of *Catla catla* Fingerlings Fed Phytase Supplemented MOSM Based Diet. *Pakistan Journal of Zoology*, 50(5). DOI:10.17582/journal.pjz/2018.50.5.1819.1828

Shakir, A., Chaudhry, A.S. and Qazi, J.I., 2013. Impact of anthropogenic activities on physico-chemical parameters of water and mineral uptake in *Catla catla* from river Ravi, Pakistan. *Environmental monitoring and assessment*, 185, 2833-2842. DOI:10.1007/s10661-012-2915-3

Siddiqui, S.A., Alvi, T., Sameen, A., Khan, S., Blinov, A.V., Nagdalian, A.A. and Onwezen, M., 2022. Consumer acceptance of alternative proteins: A systematic review of current alternative protein sources and interventions adapted to increase their acceptability. *Sustainability*, 14(**22**), 15370. DOI:10.3390/su142215370

Snedecor, G.W. and Cochran, W.G., 1991. Statistical Methods. 8th Ed. Iowa State University, Americans Press, USA. 503 P.

Soetan, K.O. and Oyewole, O.E., 2009. The need for adequate processing to reduce the anti- nutritional factors in plants used as human foods and animal feeds: A review. *African Journal of Food Science*, 3(**9**), 223-232. DOI:10.5897/AJFS.9000186

Steel, R.G.D., Torrie, J.H. and Dickey, D.A., 1996. Principles and procedures of statistics (3rd ed.). McGraw Hill international Book Co. Inc., New York, USA. pp 336-352.

Wagner, E.J., Jeppsen, T., Arndt, R., Routledge, M.D. and Bradwisch, Q., 1997. Effects of rearing density upon cutthroat trout hematology, hatchery performance, fin erosion, and general health and condition. *The Progressive Fish-Culturist*, 59(**3**), 173-187. DOI:10.1577/1548-8640(1997)059

Wedemeyer, G.A. and Yastuke, W.T., 1977. Clinical methods for the assessment of the effects of environmental stress on fish health. United States Fish and Wildlife Service Technical papers, USA. 89 P.

Ye, J., Liu, X., Wang, Z. and Wang, K., 2011. Effect of partial fish meal replacement by soybean meal on the growth performance and biochemical indices of juvenile Japanese flounder *Paralichthys olivaceus*. *Aquaculture International*, 19, 143-153. DOI:10.1007/s10499-010-9383-5

Yoo, G. and Bai, S.C., 2014. Effects of the dietary microbial phytase supplementation on bioavailability of phosphorus in juvenile olive flounder *Paralichthys olivaceus* fed soybean meal based diets. *Fish Aquaculture Science,* 17, 319-324. DOI:10.5657/FAS.2014.0319

Zhu, Y., Qiu, X., Ding, Q., Duan, M. and Wang, C., 2014. Combined effects of dietary phytase and organic acid on growth and phosphorus utilization of juvenile yellow catfish *Pelteobagrus fulvidraco*. *Aquaculture*, 430, 1-8. DOI:10.1016/j.aquaculture.2014.04.007

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