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REPLACING FISH MEAL BY CANOLA MEAL AND SUPPLEMENTING WITH PHYTASE AND CITRIC ACID FOR IMPROVING MINERAL DIGESTIBILITY IN *Cirrhinus mrigala* FINGERLINGS

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ABSTRACT

The purpose of this study was to analyze how the mineral digestibility of *Cirrhinus mrigala* was improved by the replacement of canola meal (CM) along with the supplementation of citric acid (CA) and phytase (PHY). A 90-day feeding trial was conducted in a completely randomized design. Sixteen test diets were formulated with different concentrations of CM (0%, 25%, 50%, and 75%), in the basal diet. Each concentration level was supplemented with four doses, one without supplementation, second with 2.5% CA, third with 750 FTUkg^{-1} PHY and fourth with combined supplementation (CA + PHY) to form sixteen test diets (T_1-T_{16}). Fifteen fingerlings (N = 720) were kept in each tank in triplicate. At the end of the trial, it was revealed that as the amount of CM in the diets increased, there was a significant ($P \leq 0.05$) increase in mineral digestibility (Fe, Ca, Mg, K, Mn, Cu, P, Na, Cr, Zn and Al). The fish that consumed 50% CM-based diet supplemented with 2.5% CA and 750 FTUkg^{-1} PHY had the maximum apparent digestibility coefficient of minerals (K 73.76%, Ca 64.79%, Na 62.56%, P 73.11%, Cu 76.52%, Fe 77.14%, Mn 71.87%, Mg 71.42%). The results of this research suggest that supplementing a diet high in CM (50%) with 2.5 percent CA and 750 FTUkg^{-1} PHY is the most effective strategy to increase the digestibility of minerals in *C. mrigala* fingerlings.

Keywords: Fish meal, substitution, aquaculture, plant meal, acidified phytase

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INTRODUCTION

The Indian major carp family includes *Cirrhinus mrigala,* which has aquaculture potential and consumer demand; making it a valuable resource for both business and consumers (Mayank and Dwivedi, 2015). Fish feed accounts for around 60% of a fish's total market value. However, one of the most significant protein source is fish meal because it contains the necessary amino acids and other minerals, but it is quite costly. Thus, many researchers are looking for alternatives to the high cost and limited availability of fish meal as a source of fish feed protein (Habib *et al*., 2018).

One of the most promising fish feed options is canola meal (CM), which contains 38% protein and is low in anti-thyroid factors (Belo, 2013). Moreover, CM is easily accessible and more economical than fish meal (Kaiser *et al*., 2022). Compared to soybean meal (SBM), it is the second most commonly traded protein component (Ash *et al*., 2006; Abakari and Wu, 2015). Anti-nutritional elements such as fibres, glucosinolates, phytic acids, synaptic acids and tannins that restrict usage of CM, are present in significant concentrations (von Danwitz and Schulz, 2020).

Phytate, for example, is an anti-nutritional chemical, available in CM, that should be avoided at all costs. Toxic levels of phytate in CM can harm fish gut health and stunt growth, making it an undesirable food source for fish (Usmani and Jafri, 2002). Fish like *C. mrigala* cannot make their own phytase enzyme (Ogino *et al*., 1979). Therefore, negatively charged ion phytate forms insoluble complexes with cations like Fe^{+3}, Mn^{+2}, Fe^{+2}, Ca^{+2}, Zn^{+2}, and Cu^{+2}. Poor phytate breakdown exacerbates eutrophication, which is hazardous to freshwater systems (Persson *et al*., 1998; Marolt *et al*., 2020). Phosphate is removed from phytate by microbial phytase (PHY), which makes it usable and absorbable for fish (Baruah *et al*., 2007; Kumar *et al*., 2012; Sarfraz *et al*., 2020). The supplementation of PHY improved the mineral digestibility in rainbow trout (Sugiura *et al*., 2001).

Furthermore, PHY action is pH dependent, even though it is activated to a large extent at low gut pH (Baruah *et al*., 2007). Carnivore fishes can produce acid, which lowers the pH of the stomach, while monogastric fishes do not have this ability (Ogino *et al*., 1979). Citric acid (CA) is an organic acid and is widely recognized for lowering the pH of the gut and improving PHY effectiveness in fish feed (Baruah *et al*., 2005). Additionally, PHY enhances the availability of P and other minerals by dephosphorylating the phytate complex, thus creating the optimum conditions for CA (Zyla *et al*., 1995). The Ca and P levels of Beluga (*Huso huso*) were significantly increased by adding CA to their diet (Khajepour *et al*., 2011). Moreover, CA in fish diets enhanced the absorption of phosphorus and other minerals (Sugiura *et al*., 2001). In this study, the hypothesis is to determine whether *C. mrigala* fingerlings, when fed with different levels of CM as replacement of fish meal along with the PHY and CA supplementation, both separately and together, positively affected the mineral digestibility.

MATERIALS AND METHODS

Experimental diets: Sixteen experimental diets were prepared in Laboratory of the Government College University, Faisalabad, Pakistan at the latitude: 31°24'32.21" N, longitude: 73°04'5.86" E and altitude 180 m. The chemical composition of all the experimental diets were similar, apart from CA and PHY supplementation (Table 1). CA and PHY were supplemented to experimental diets at concentrations of 0% and 2.5% and 0 and 750 FTUkg^{-1}, respectively. A grinding machine was used for grinding all feed ingredients down to a 0.05 mm particle size. Gradually, fish oil was added for binding then CA was also added in it and blended electrically. The dough was prepared by adding 15% water, which was then processed to form a suitable dough. The pellets were then sprayed with two different concentrations of PHY (0 and 750 FTUkg^{-1}) according to method of Robinson *et al*. (2002). The pellets were air-dried and then placed in airtight plastic jars and stored at -20°C during the feeding investigation. A completely randomized design was synthesized in triplicates.

Rearing conditions and experimental fish setup: *C. mrigala* fingerlings (6.35±0.04 g/fish) were procured from the Government Fish Seed Hatchery Faisalabad. When fish were transferred to laboratory, they were bathed in salt solution (5g/L NaCl). During the acclimatization phase, the basal diet was added every day to each test tank which also had aeration going on all the time. After a 2 week acclimatization period, 15 fingerlings were randomly selected for each of 48 experimental tanks, each having capacity of 70 L of water. The fingerlings were 5% wet body weight twice a day (at 9:00 am and 2:00 pm). The experimental tanks were cleaned and replenished with water after 2 hours of feeding. During the 90-day feeding study, the physicochemical parameters such as average water temperature (24–26°C), pH (7.6–8.0), and amount of dissolved oxygen (DO 5–6 mg/L) were maintained regularly.

Collection of sample and analysis: Throughout the trial, after a two-hour feeding session, the fecal collecting tubes were used for fecel collection, then uneaten diet was removed from all experimental tanks by opening the valves. After washing the diet particles out of the tanks, water was added again. However, to reduce nutrient leakage, fecal threads were taken carefully. Fecal material was oven dried at 60°C from each replicated treatment, crushed, and kept for chemical analysis. Following established protocols (AOAC, 2005), perchloric acid and nitric acid mixture (2:1) digested the diet and feces samples for mineral estimation. Mineral concentrations such as magnesium (Mg), copper (Cu), zinc (Zn), manganese (Mn), iron (Fe), and calcium (Ca) were evaluated using atomic absorption techniques after suitable dilution (Hitachi Polarized Atomic Absorption Spectrometer, Z-8200). Available commercial standards (AppliChem® Gmbh Ottoweg4, DE- 64291 Darmstadt, Germany) were used to produce calibrated standards for mineral estimation. A flame photometer was used to quantify sodium (Na) and potassium (K) samples (Jenway PFP-7, UK). Using standard technique, the phosphorus (P) sample was evaluated calorimetrically (UV/VIS spectrophotometer) with ammonium molybdate (720 nm absorbance) (AOAC, 2005). Apparent digestibility coefficient (ADC%) was evaluated by following the protocol of Hussain *et al*. (2018).

Statistical analysis: The CoStat software was used to assessed Three-way Analysis of Variance (ANOVA) on the experimental data in order to determine the effectiveness of CM based diets along with CA and PHY supplementation as well as the interaction between them (Version 6.303, Monterey, CA, 93940, PMB 320 USA). Moreover, Tukey's Honestly Significant Difference Test (Snedecor and Cochran, 1989) was used to compare means at ($P \leq 0.05$).

RESULTS

The results of mineral composition in feed, feces and ADC% in *C. mrigala* fingerlings are shown in Tables 2, 3 and 4, respectively. The mineral composition in diets of *C. mrigala* supplemented with CA and PHY was statistically similar. All the CM based diets along with the supplementation of CA and PHY significantly enhanced the mineral composition in feces of *C. mrigala*. In comparison to fish fed on a diet without CA and PHY, the mineral availability was not improved. On the other hand, CA and PHY supplementation indicated improved ADC% of all the measured minerals. When compared to T_1, ADC% showed that adding CA and PHY in fish diet significantly ($P \le 0.05$) increased mineral digestibility. CA 2.5% and PHY 750 FTU kg^{-1} (T_{12}) increased the digestion of Fe, K, Ca, Cu, P, Mn, Mg and Al. Maximum ADC% of Ca (64.79%), K (73.76%), Fe (77.14%), Cu (76.52%), Mn (71.87%), P (73.11%), Mg (71.42%) and Al (66.51%) were found in diets supplemented with 2.5% CA and 750 FTU kg^{-1} along with 50% CM based diet. It was shown that making the diet more acidic with CA made it much easier to digest the minerals in the phytase-treated diet. The T_{13} and T_{16} diet groups had the highest concentrations of Zn (79.30%), Na (63.51%), and Cr (62.69%).

Table 1. Diet composition (%) of experimental diets.

Test diets	Fish meal Replacement with CM (%)	Fishmeal	Canola meal	PHY (FTU kg^{-1})	CA (%)	Rice polish	Wheat flour	Fish oil	*Vitamins Premix	**Minerals Premix	Chromic Oxide
T_1	0%	48	18	0	0	3	22	6	1	1	1
T_2		48	18		2.5	3	19.5	6	1	1	1
T_3		48	18	750	0	3	22	6	1	1	1
T_4		48	18		2.5	3	19.5	6	1	1	1
T_5	25%	36	35	0	0	3	17	6	1	1	1
T_6		36	35		2.5	3	14.5	6	1	1	1
T_7		36	35	750	0	3	17	6	1	1	1
T_8		36	35		2.5	3	14.5	6	1	1	1
T_9	50%	24	53	0	0	3	11	6	1	1	1
T_{10}		24	53		2.5	3	8.5	6	1	1	1
T_{11}		24	53	750	0	3	11	6	1	1	1
T_{12}		24	53		2.5	3	8.5	6	1	1	1
T_{13}	75%	12	71	0	0	3	5	6	1	1	1
T_{14}		12	71		2.5	3	2.5	6	1	1	1
T_{15}		12	71	750	0	3	5	6	1	1	1
T_{16}		12	71		2.5	3	2.5	6	1	1	1

*Vitamin (Vit.) premix kg^{-1}: Vit. A: 15,000,000 IU, Vit. C: 15,000 mg, Vit. E:30000 IU, Vit. B_2: 7000 mg, Vit. B_6: 4000 mg, Vit. B_{12}: 40 mg, Vit. D_3: 3,000,000 IU, Vit. K_3: 8000 mg, Ca pantothenate: 12,000 mg, Nicotinic acid: 60,000 mg, Folic acid: 1500 mg

**Mineral premix kg^{-1}: P: 135 g, Fe: 1000 mg, Mg: 55 g, Na: 45 g, Zn: 3000mg, Co: 40 mg, Ca: 155 g, I: 40 mg, Mn: 2000 mg, Se: 3 mg, Cu: 600 mg

Table 2. Analyzed mineral composition (%) in diets of *C. mrigala* fed with CA and PHY supplementation.

Test diets	Fish meal Replacement Levels (%)	PHY	CA	Ca	Cr	K	Mn	Fe	Cu	Zn	Al	Na	Mg	P
T_1		0	0	3.20	0.032	0.260	0.017	0.019	0.0113	0.029	0.00018	0.24	0.0055	0.515
T_2	0%		2.5	3.20	0.031	0.263	0.012	0.013	0.0115	0.029	0.00018	0.21	0.0054	0.515
T_3		750	0	3.21	0.031	0.267	0.012	0.012	0.0121	0.029	0.00018	0.23	0.0051	0.515
T_4			2.5	3.16	0.033	0.267	0.012	0.012	0.0124	0.029	0.00018	0.24	0.0055	0.516
T_5		0	0	3.15	0.028	0.250	0.012	0.012	0.0122	0.029	0.00018	0.24	0.0221	0.513
T_6	25%		2.5	3.11	0.029	0.257	0.012	0.012	0.0122	0.029	0.00042	0.27	0.0055	0.514
T_7		750	0	3.12	0.025	0.253	0.012	0.012	0.0125	0.028	0.00018	0.26	0.0051	0.514
T_8			2.5	3.16	0.024	0.250	0.012	0.012	0.0117	0.029	0.00018	0.23	0.0058	0.515
T_9		0	0	3.15	0.029	0.237	0.012	0.012	0.0118	0.029	0.00018	0.24	0.0055	0.514
T_{10}	50%		2.5	3.17	0.029	0.250	0.012	0.012	0.0113	0.028	0.00018	0.27	0.0053	0.543
T_{11}		750	0	3.14	0.037	0.253	0.012	0.012	0.0125	0.028	0.00018	0.25	0.0056	0.545
T_{12}			2.5	3.18	0.032	0.247	0.012	0.012	0.0146	0.029	0.00018	0.22	0.0056	0.541
T_{13}		0	0	3.14	0.032	0.257	0.012	0.012	0.0116	0.029	0.00018	0.23	0.0055	0.552
T_{14}	75%		2.5	3.16	0.029	0.243	0.012	0.012	0.0118	0.029	0.00018	0.28	0.0056	0.543
T_{15}		750	0	3.14	0.015	0.247	0.012	0.012	0.0114	0.029	0.00018	0.24	0.0057	0.512
T_{16}			2.5	3.13	0.036	0.253	0.012	0.012	0.0163	0.029	0.00018	0.25	0.0057	0.531
Pool Standard Error (PSE)				0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

The data represent the mean of three replicates. Means within columns with distinct superscripts exhibit significant difference ($P \leq 0.05$).

Table 3. Analyzed mineral composition (%) in feces of *C. mrigala* fed with CA and PHY supplementation.

Test diets	Fish meal Replacement Levels (%)	PHY	CA	Ca	Cr	K	Mn	Fe	Cu	Zn	Al	Na	Mg	P
T_1		0	0	1.413^{c}	0.011^{f}	0.117^{c}	0.0056^{c}	0.00387^{c}	0.0042^{a}	0.011^{b}	0.0001^{a}	0.102^{g}	0.0038^{c}	0.261^{i}
T_2	0%		2.5	1.180^{g}	0.010^{f}	0.076^{h}	0.0050^{d}	0.00453^{c}	0.0037^{a}	0.010^{b}	0.0001^{a}	0.099^{h}	0.0029^{c}	0.287^{h}
T_3		750	0	1.416^{c}	0.006^{g}	0.096^{g}	0.0055^{c}	0.00528^{c}	0.0049^{a}	0.009^{b}	0.0001^{a}	0.111^{d}	0.0025^{c}	0.228^{m}
T_4			2.5	1.590^{b}	0.017^{e}	0.111^{f}	0.0067^{b}	0.00574^{c}	0.0058^{a}	0.013^{b}	0.0001^{a}	0.119^{b}	0.0053^{b}	0.342^{d}
T_5		0	0	1.856^{a}	0.026^{c}	0.147^{a}	0.0077^{a}	0.00685^{b}	0.0060^{a}	0.018^{a}	0.0002^{a}	0.133^{a}	0.0047^{c}	0.330^{e}
T_6	25%		2.5	1.403^{c}	0.027^{c}	0.117^{c}	0.0055^{c}	0.00383^{c}	0.0044^{a}	0.011^{b}	0.0002^{a}	0.103^{g}	0.0033^{c}	0.258^{j}
T_7		750	0	1.270^{f}	0.009^{f}	0.076^{h}	0.0049^{d}	0.00434^{c}	0.0036^{a}	0.010^{b}	0.0001^{a}	0.093^{j}	0.0026^{c}	0.287^{h}
T_8			2.5	1.323^{e}	0.011^{f}	0.096^{g}	0.0055^{c}	0.00528^{c}	0.0044^{a}	0.009^{b}	0.0001^{a}	0.106^{f}	0.0023^{c}	0.232^{l}
T_9		0	0	1.570^{b}	0.114^{b}	0.111^{f}	0.0060^{c}	0.00520^{c}	0.0047^{a}	0.013^{b}	0.0001^{a}	0.117^{c}	0.0061^{b}	0.327^{f}
T_{10}	50%		2.5	1.850^{a}	0.022^{d}	0.147^{a}	0.0078^{a}	0.00612^{b}	0.0048^{a}	0.018^{a}	0.0001^{a}	0.133^{a}	0.0064^{b}	0.347^{c}
T_{11}		750	0	1.410^{c}	0.011^{f}	0.117^{c}	0.0066^{b}	0.00367^{c}	0.0046^{a}	0.012^{b}	0.0002^{a}	0.104^{g}	0.0055^{b}	0.213^{n}
T_{12}			2.5	1.193^{g}	0.010^{f}	0.076^{h}	0.0057^{c}	0.00787^{a}	0.0067^{a}	0.010^{b}	0.0001^{a}	0.093^{j}	0.0039^{c}	0.184^{o}
T_{13}		0	0	1.370^{d}	0.011^{f}	0.096^{g}	0.0048^{d}	0.00504^{c}	0.0042^{a}	0.0098^{b}	0.0001^{a}	0.097^{i}	0.0021^{c}	0.291^{g}
T_{14}	75%		2.5	1.563^{b}	0.139^{a}	0.115^{e}	0.0059	0.00520^{c}	0.0039^{a}	0.013^{b}	0.0001^{a}	0.108^{e}	0.0087^{a}	0.352^{b}
T_{15}		750	0	1.886^{a}	0.025^{c}	0.144^{b}	0.0068^{b}	0.00512^{c}	0.0055^{a}	0.018^{a}	0.0002^{a}	0.103^{g}	0.0059^{b}	0.353^{a}
T_{16}			2.5	1.406^{c}	0.011^{f}	0.116^{d}	0.0076^{a}	0.00293^{d}	0.0043^{a}	0.013^{b}	0.0001^{a}	0.132^{a}	0.0064^{b}	0.256^{k}
Pool Standard Error (PSE)				0.04	0.04	0.01	0.01	0.01	0.01	0.01	0.04	0.01	0.01	0.01

The data represent the mean of three replicates. Means within columns with distinct superscripts exhibit significant difference ($P \leq 0.05$).

Table 4 Analyzed mineral digestibility (%) of *C. mrigala* fed with CA and PHY supplementation.

Test diets	Fish meal Replacement Levels (%)	PHY	CA	Ca	Cr	K	Mn	Fe	Cu	Zn	Al	Na	Mg	P
T_1	0%	0	0	53.51^d	42.14^c	52.95^e	42.98^g	60.53^b	53.15^d	60.14^e	46.42^c	43.17^d	54.66^e	44.65^f
T_2			2.5	62.79^b	47.55^c	59.43^c	51.87^d	54.14^c	59.85^c	65.30^c	56.51^b	54.28^c	56.42^d	51.08^d
T_3		750	0	61.61^b	52.71^b	55.79^d	48.92^e	52.85^c	50.71^e	66.30^c	48.39^c	47.66^d	51.69^f	52.02^d
T_4			2.5	53.21^d	45.88^c	49.45^f	40.15^h	49.70^c	45.40^f	60.09^e	43.94^c	45.88^d	48.97^g	43.41^f
T_5	25%	0	0	44.22^f	43.07^c	43.13^h	33.74^i	38.08^c	41.46^g	44.005^j	38.65^d	46.35^d	47.67^h	45.13^f
T_6			2.5	52.50^d	48.14^c	46.95^g	46.31^f	50.38^c	43.14^g	57.81^f	46.09^c	47.17^d	54.63^e	45.32^f
T_7		750	0	63.12^b	55.55^b	61.76^b	52.54^d	47.14^c	49.85^e	62.97^d	52.51^c	53.23^c	55.62^d	52.35^d
T_8			2.5	59.61^c	48.71^c	56.46^d	53.59^d	42.85^c	40.71^g	65.30^c	48.39^c	51.66^c	51.22^f	47.32^e
T_9	50%	0	0	51.88^d	53.88^b	49.78^f	50.48^d	46.37^c	39.24^g	52.75^h	43.26^c	50.21^c	51.61^f	41.47^f
T_{10}			2.5	48.22^e	50.07^c	44.13^h	53.74^d	41.42^c	34.79^h	46.00^i	46.65^c	44.69^d	37.37^k	42.39^f
T_{11}		750	0	52.50^d	48.81^c	52.62^e	62.98^b	50.53^c	43.13^g	35.47^k	58.42^b	42.51^d	64.59^c	62.94^b
T_{12}			2.5	64.79^a	44.88^c	73.76^a	71.87^a	77.14^a	76.52^a	36.64^k	66.51^a	62.56^a	71.42^a	73.11^a
T_{13}	75%	0	0	61.61^b	48.38^c	62.79^b	52.26^d	42.85^c	47.38^f	79.30^a	39.72^d	47.66^d	51.68^f	51.45^d
T_{14}			2.5	49.21^e	45.67^c	58.12^c	53.81^d	46.37^c	42.57^g	72.75^b	35.60^d	43.88^d	45.93^i	41.57^f
T_{15}		750	0	42.89^g	48.85^c	53.40^e	57.07^c	41.42^c	38.15^g	54.68^g	33.31^d	54.35^c	41.51^j	43.13^f
T_{16}			2.5	52.83^d	62.69^a	52.28^e	57.64^c	53.86^c	63.39^b	52.14^b	46.42^c	63.51^a	66.90^b	58.98^c
Pool Standard Error (PSE)				1.37	1.93	1.62	1.41	1.59	2.09	1.63	2.28	1.19	0.87	1.59

The data represent the mean of three replicates. Means within columns with distinct superscripts exhibit significant difference ($P \leq 0.05$).

DISCUSSION

Fish meal was traditionally used to manufacture fish feed due to its high contents of important nutrients and minerals. Plant by products are increasingly being added in fish diets as an alternate source of protein. Due to binding between essential minerals and the phytate complex, they contain an anti-nutritional component called phytate, which renders nutrients inaccessible to fish and reduces their absorption (Soetan and Oyewole, 2009; Hussain *et al*., 2011; Liu *et al*., 2013; Hussain *et al*., 2014; Dersjant-Li *et al*., 2015; Hussain *et al*., 2015; Selamoglu, 2018; Sarfraz *et al*., 2020).

Our results showed that when *C. mrigala* was fed on T_{12} diet, the fish digested improved level of minerals i.e. Fe (77.14%), Ca (64.79%), Mg (71.42%), K (73.76%), Mn (71.87%), Cu (76.52%), P (73.11%), and Al (66.51%). On the other hand, the highest Zn levels were found in *C. mrigala* that were fed 75% replacement diet without any supplementation of CA or PHY. The presence of dietary phytic acid may cause moderate variation in the digestion of minerals i.e. Zn, Cu, K, and Na. Moreover, Rasid *et al*. (2021) revealed that when developing fish diets, special consideration should be given to PHY. In the same way, Shah *et al*. (2021) found less discharge of minerals through feces when *Labeo rohita* were fed SBM containing diet with 3% CA and 1000 FTU/kg PHY level. In accordance to our results, Hussain *et al*. (2018) found that *C. mrigala* fingerlings with the highest ADC% of minerals (Zn 74%, Na 64%, Cu 68%, Fe 64%, Ca 68%, P 77%, Mg 53%, K 62%, and Mn 67%) when fed with corn gluten meal based diet (30%) supplemented with 5% CA and 500 $FTUkg^{-1}$ PHY. They hypothesized that the chelated minerals may be released and made more readily available to fish by the usage of CA and PHY in diets.

Rabia *et al*. (2017) found that the mineral digestibility of Ca, Fe, K, Cu, P, and Na was significantly ($P \leq 0.05$) enhanced when *L. rohita* fed a diet based on SFM supplemented with 1000 $FTUkg^{-1}$ of PHY and 2% CA. As a result, there was less pollution in the aquatic environment when CA and PHY were combined in a plant-based diet. The *C. mrigala* fingerlings grow more quickly as a result of the increased availability of minerals. Similar findings were presented on *L. rohita* (Shah *et al*., 2015; Afzal *et al*., 2019, 2020; Shah *et al*., 2021) and *C. mrigala* (Baruah *et al*., 2007; Hussain *et al*., 2018) that their mineral absorption was significantly improved ($P \leq 0.05$) by CA and PHY supplementation.

Our study found that when fish were fed control diet, their mineral digestibility was lower as compared to the CA and PHY treated groups. The absorption of other minerals, such as Al, Ca, Mg, Cu, K, Mn and Fe were found to increase when phosphorus digestibility was enhanced. Hence, a positive correlation between CA and PHY and mineral absorption can be observed. PHY may have been able to function optimally in the gut due to the presence of CA, which may have resulted in a more synergistic effect on mineral digestion. Diet acidification has also been shown to slow stomach emptying, allowing more time for PHY to function (Shah *et al*., 2015). In line with our study, Habib *et al*. (2018) reported that CM based diets along with CA and PHY supplementation significantly improved the mineral digestibility. Mineral absorption was also improved in rainbow trout and rohu when fed with CA and PHY supplemented plant-based by-products by Sugiura *et al*. (2001) and Baruah *et al*. (2007), respectively. Variations in outcomes are due to different feeding habitats, species, size, plant ingredients, diet composition and environmental conditions (Duran and Talas, 2009).

Conclusion: The CA (2.5%) and PHY (750 $FTUkg^{-1}$) supplementation increased the mineral utilization of *C. mrigala* fed a diet of plant proteins (50% CM-based diet). Individually, this improvement was attained, and it was further enhanced by working together. The supplements showed that it might be possible to make cheap feed that is also good for the environment by reducing the amount of minerals that are used and then dumped into water bodies.

Conflicts of Interest: The authors declare that there is no conflict of interests regarding the publication of this paper.

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Data availability statement: All data related to this experiment is available from the corresponding author upon reasonable request.

Ethical statement: Ethical approval is not required for this study.

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