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Dietary Supplementation of Citric Acid and Phytase in Plant-Based Diets Improves Mineral Bioavailability in Common Carp, *Cyprinus carpio*

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ABSTRACT

The common carp (*Cyprinus carpio*) is an agastric fish, having digestive tract pH above 6 and cannot digest phytate properly found in plant-based diets. Phytate acts as an anti-nutrient, as it chelates with minerals to form insoluble mineral-phytate complexes and reduce their bioavailability. Therefore, in this study citric acid (CA) and exogenous phytase (PHY) were incorporated with canola meal-based (CM) diets to check their effects on mineral availability and digestibility in *C. carpio* fingerlings. Four CM-based diets were prepared by substituting CM for fishmeal at 0, 25, 50, and 75% levels, in which PHY (0 and 750 FTU kg^{-1}) and CA (0 and 2.5%) were added at two levels, creating a total of sixteen test diets labelled from T_1 to T_{16}. For 8-week, common carp were fed on test diets about 2.5% of their live body weight. Fecal samples were collected from each tank twice daily to assess mineral digestibility. The results showed that the highest apparent digestibility coefficients for minerals were observed in fish fed on T_{12} diet (50% CM, 2.5% CA and 750 FTUkg^{-1} PHY), This combination synergistically improved the digestibility of minerals, such as calcium (63%), phosphorus (70%), sodium (60%), potassium (70%), magnesium (68%), iron (73%), copper (73%), manganese (67%), and zinc (63%). These findings suggested that supplementing CM based diets with CA and PHY effectively released chelated minerals from phytate complexes, thereby enhancing their availability for *C. carpio*. This approach can contribute to improve fish performance and overall nutritional efficiency. Incorporating CA and PHY in CM based diets holds promise for optimizing mineral digestibility in fish.

INTRODUCTION

The common carp (*Cyprinus carpio*) is a significant economic fish species that is well known for its superior nutrition, fastest growth rate, and long standing breeding history. *C. carpio* is an omnivorous bottom feeder carp that feeds on plants, small invertebrates as well as detritus (Adámek *et al*., 2019; Selamoglu *et al*., 2015). It is a major contributor to freshwater aquaculture (Nakajima *et al*., 2019). In 2018, the production of common carp made up 7.7% (4.2 Mt) of all fish produced globally (Eljasik *et al*., 2022). Carp aquaculture was predicted to consume 27% (13.5 Mt) of aqua-feed globally, in 2015. Around 37.5% (5.1 Mt) of this aqua-feed intended for carp farming worldwide was consumed by common carp alone (FAO FishStat, 2017). Fishmeal is considered as an ideal source of protein in aqua-feed owing to its well-balanced amino acids, fatty acids and mineral content. It has less carbohydrates and anti-nutrients with higher palatability as well as digestibility (Gatlin *et al*., 2007). Over the years, expensive fishmeal inclusion

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rates have significantly reduced in feed based aquaculture industry (Olsen and Hasan, 2012), that resulted in extensive research and usage of different plant protein sources.

Canola meal (CM), a plant protein source, has significant potential to replace FM. It is a by-product of the oil-processing industry, having comparatively higher protein level (~ 38%), a balanced amino acid composition and lower production cost (Enami, 2011). However, there are number of anti-nutritional factors in CM i.e., fiber, glucosinolates, phenolic compounds and phytic acid that limit its use, particularly in animal nutrition. Phytate or phytic acid, a reservoir of phosphorus, is found in CM about 4-5% and lower its nutritive value by making bonds with multivalent cations i.e., Zn, Fe and Ca and hence, lowering their availability to animals (Al-Asheh and Duvnjak, 1994; Sarfraz *et al*., 2020).

Microbial phytase (PHY) separates minerals and phosphorus, lowering inositol phosphate from phytate. Monogastric and agastric animals show natural deficiency of PHY due to absence of PHY producing microbes. Therefore, the dietary incorporation of exogenous PHY to plant-based fish diet improves the minerals bioavailability and digestibility, which results in less nutrient loss through excretion in the feces, reducing water body pollution (Priya *et al*., 2023). PHY needs an optimum range pH (2.5-5.5) to function effectively. Fish without stomachs, such as the common carp, have digestive systems with neutral to alkaline pH. This huge pH difference between the optimum PHY activity and intestine of agastric fish is a main reason of poor efficiency of PHY (Kumar *et al*., 2012; Sarfraz *et al*., 2020).

Organic acids i.e., citric acid (CA) can be used as feed additive to increase the efficiency and utilization of PHY because these organic acids reduce the acidity of feed, lower the digestive tract pH and boost the efficiency of supplemented PHY (Baruah *et al*., 2005; Kumar *et al*., 2012). Decrease in pH by organic acids may directly increase the phytate solubility, and dissociate mineral complexes, improving mineral availability and absorption in gut (Canibe *et al*., 2005). PHY and CA has been proposed as promising feed additive to improve mineral availability and digestibility, improving general fish health. Therefore, the present research work aimed to examine the individual and synergistic impacts of dietary PHY and CA on the digestibility and absorption of minerals present in CM based diets of *C. carpio*, which is a significant aquaculture fish species worldwide.

MATERIALS AND METHODS

Fish and experimental conditions

The *C. carpio* fingerlings were obtained from the Government Fish Hatchery in Faisalabad. Prior to start of the trial, a disinfection process was conducted on the fingerlings. This involved immersing them in a sodium chloride (NaCl) solution with a concentration of 5g/L for a specific duration. After the disinfection process, fingerlings were shifted to cement tanks for two weeks for acclimatization, fed on basal diet once daily. Throughout the experiment, water quality indicators i.e., temperature, pH, and dissolved oxygen were carefully monitored regularly.

Feed ingredients and test diets

All ingredients were sourced from the local commercial feed market. Prior to formulation, the chemical composition of these feed ingredients was analyzed using standard methods provided by AOAC (2005). CM, the basic test ingredient, was used to replace fishmeal at levels of 0, 25, 50, and 75%, resulting four CM based test diets, in which PHY (0 and 750 FTU kg-1) and CA (0% and 2.5%) at two levels were incorporated, resulting in a total of sixteen test diets labeled from T_1 to T_{16} (Table I). After undergoing the grinding process, the feed ingredients were sieved through a mesh with a size of 0.5mm to ensure a consistent particle size. Subsequently, these ingredients were mixed in a food mixer for approximately 5 min. During the mixing process, fish oil was added gradually to mixture. After that to form a suitable dough, about 10-15% water was added. Pellets were formed via pelleting machine, then dried and placed at 4°C to be used in trial.

Plan of feeding and sample collection

Each experimental diet was tested in triplicates (16 experimental diets × 3 tanks). Each tank was initially stocked with 15 fingerlings. The feeding schedule was two times a day (at 8:00 am and 2:00 pm). The fingerlings were given their referred diets, ~ 5% of their live body weight. Following 2h. of feeding, fecal matter was collected via specially designed V-shaped water tanks. Great care was taken during fecal collection to avoid the breakdown of fecal strings and nutrient leaching. The fecal material collected from the tanks was carefully dried in an oven set at 60°C to remove moisture content. Subsequently, the dried fecal material was finely ground into a powder to ensure homogeneity and facilitate chemical analysis. The powdered samples were then stored in the laboratory under appropriate conditions to maintain their integrity for future analysis.

Mineral analysis of feed and feces

Before analysis, test diets and feces samples were individually homogenized using a mortar and pestle to ensure consistency. The analysis was done by following standard procedures outlined by AOAC (2005). To estimate

Table I. Ingredients composition (%) of experimental diets.

Test diets	Fishmeal replacement levels (%)	Fish-meal	Canola meal	Phytase (FTU kg^{-1})	Citric acid (%)	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

Wheat flour was substituted for phytase and citric acid. *Vitamin (Vit.) premix kg-1: Vit. A: 15,000,000 IU, Vit. B12: 40 mg, Vit. C: 15,000 mg, Vit. D3: 3,000,000 IU, B2: 7000 mg, Vit. E:30000 IU, Vit. B6: 4000 mg, Vit. Ca pantothenate: 12,000 mg, Vit. K3: 8000 mg, Folic acid: 1500 mg, Nicotinic acid: 60,000 mg. **Mineral premix kg-1: Fe: 1000 mg, Ca: 155 g, P: 135 g, Se: 3 mg, Na: 45 g, Co: 40 mg, Zn:3000mg, Cu: 600 mg, Mn: 2000 mg, I: 40 mg, Mg: 55 g.

mineral content, the samples were digested individually in a mixture of perchloric acid and boiling nitric acid (1:2 ratios). Following dilution using distilled water, mineral content was evaluated using an Atomic Absorption Spectrophotometer (Hitachi Polarized Atomic Absorption Spectrometer, Z-8200). Calibrated standards formed with commercially accessible standards from AppliChem® Gmbh Ottoweg4, DE-64291 Darmstadt, Germany, were used for estimating mineral contents. For phosphorus content, a UV/VIS spectrophotometer at 720nm absorbance was used for calorimetric assessment via ammonium molybdate reagent. Sodium and potassium content was measured utilizing a flame photometer (Jenway PFP-7, UK). The chromic oxide, an inert marker, in test diets and feces was estimated by Spectrophotometer at 370nm absorbance after oxidation via perchloric acid (Divakaran et al., 2002).

Calculation of apparent mineral digestibility coefficients

The apparent digestibility coefficients (ADC%) of the minerals in diets were estimated by the following standard formula (NRC, 1993):

ADC (%) = [100- (100 × (Percentage marker in diet × Percentage nutrient in feces)/ (Percentage marker in feces × Percentage nutrient in diet))]

Statistical analysis

One way analysis of variance (ANOVA) was utilized for analysis of data on mineral digestibility and absorption. To determine significant variations, Tukey's honestly significant difference test was applied, with a significance level set at $P<0.05$ (Snedecor and Cochran, 1989). For the statistical assay, the Co-Stat Computer Package of a version 6.303, PMB 320, Monterey, CA, 93940 USA) was utilized.

RESULTS

Significant differences in mineral compositions between the test diets and feces samples were discovered by their analysis. Tables II, III, and IV outline the primary effects of PHY, CA, and their interaction on the minerals composition of test diets, feces and minerals digestibility in *C. carpio* fingerlings. The mineral digestibility of *C. carpio* fingerlings significantly improved ($P<0.05$) with the incorporation of CA and PHY in CM based diets. In comparison, the T_{12} diet showed the maximum values of mineral digestibility with notable percentages recorded for Ca (63.12%), P (70.78%), Na% (60.56%), K (70.76%), Mg (68.07%), Fe (73.14%), Cu (73.18%), Mn (67.19%), and Zn (63.29%), which were significantly ($P<0.05$) higher

Table II. Effect of dietary supplementation of citric acid and phytase in plant based diet on mineral composition (%) of *Cyprinus carpio*.

Test diets	Fish meal replace-ment levels (%)	Phytase (FTU kg^{-1})	Citric acid (%)	Ca	Na	K	Fe	Cu	Zn	Mn	P	Mg	Al	Cr
T_1	0%	0	0	3.20	0.24	0.260	0.019	0.0113	0.029	0.017	0.515	0.0055	0.00018	0.032
T_2			2.5	3.20	0.21	0.263	0.013	0.0115	0.029	0.012	0.515	0.0054	0.00018	0.031
T_3		750	0	3.21	0.23	0.267	0.012	0.0121	0.029	0.012	0.515	0.0051	0.00018	0.031
T_4			2.5	3.16	0.24	0.267	0.012	0.0124	0.029	0.012	0.516	0.0055	0.00018	0.033
T_5	25%	0	0	3.15	0.24	0.250	0.012	0.0122	0.029	0.012	0.513	0.0221	0.00018	0.028
T_6			2.5	3.11	0.27	0.570	0.012	0.0122	0.029	0.012	0.514	0.0055	0.00042	0.029
T_7		750	0	3.12	0.26	0.253	0.012	0.0125	0.028	0.012	0.514	0.0051	0.00018	0.025
T_8			2.5	3.16	0.23	0.250	0.012	0.0117	0.029	0.012	0.515	0.0058	0.00018	0.024
T_9	50%	0	0	3.15	0.24	0.237	0.012	0.0118	0.029	0.012	0.514	0.0055	0.00018	0.029
T_{10}			2.5	3.17	0.27	0.250	0.012	0.0113	0.028	0.012	0.543	0.0053	0.00018	0.029
T_{11}		750	0	3.14	0.25	0.253	0.012	0.0125	0.028	0.012	0.545	0.0056	0.00018	0.037
T_{12}			2.5	3.18	0.22	0.247	0.012	0.0146	0.029	0.012	0.541	0.0056	0.00018	0.032
T_{13}	75%	0	0	3.14	0.23	0.257	0.012	0.0116	0.029	0.012	0.552	0.0055	0.00018	0.032
T_{14}			2.5	3.16	0.28	0.243	0.012	0.0118	0.029	0.012	0.543	0.0056	0.00018	0.029
T_{15}		750	0	3.14	0.24	0.247	0.012	0.0114	0.029	0.012	0.512	0.0057	0.00018	0.015
T_{16}			2.5	3.13	0.25	0.253	0.012	0.0163	0.029	0.012	0.531	0.0057	0.00018	0.036
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

At *P*<0.05, means within columns with distinct superscripts differ significantly. Data are the means of three replicas.

Table III. Effect of canola meal-based diets supplemented with phytase and citric acid on the minerals composition (%) in feces of *C. carpio* fingerlings.

Test diets	Fish meal replacement levels (%)	Phytase (FTU kg^{-1})	Citric acid (%)	Ca	Na	K	Fe	Cu	Zn	Mn	P	Mg	Al	Cr
T_1	0%	0	0	1.45^{c}	0.10^{a}	0.12^{b}	0.004^{c}	0.008^{a}	0.021^{f}	0.007^{a}	0.36^{g}	0.011^{b}	0.0005^{a}	0.021^{d}
T_2			2.5	1.22^{d}	0.13^{a}	0.08^{c}	0.008^{b}	0.004^{a}	0.044^{d}	0.006^{a}	0.38^{f}	0.003^{h}	0.0001^{a}	0.020^{d}
T_3		750	0	1.45^{c}	0.11^{a}	0.20^{a}	0.009^{b}	0.005^{a}	0.010^{j}	0.006^{a}	0.32^{j}	0.004^{g}	0.0002^{a}	0.013^{d}
T_4			2.5	1.52^{c}	0.13^{a}	0.12^{b}	0.006^{b}	0.006^{a}	0.053^{a}	0.007^{a}	0.44^{b}	0.006^{e}	0.0002^{a}	0.024^{d}
T_5	25%	0	0	1.66^{c}	0.13^{a}	0.15^{b}	0.040^{a}	0.007^{a}	0.028^{e}	0.008^{a}	0.43^{c}	0.005^{f}	0.0003^{a}	0.036^{c}
T_6			2.5	1.45^{c}	0.12^{a}	0.12^{b}	0.004^{c}	0.005^{a}	0.051^{b}	0.006^{a}	0.35^{h}	0.004^{g}	0.0006^{a}	0.037^{c}
T_7		750	0	1.32^{c}	0.10^{a}	0.08^{c}	0.038^{a}	0.004^{a}	0.020^{f}	0.006^{a}	0.38^{f}	0.003^{h}	0.0001^{a}	0.016^{d}
T_8			2.5	1.41^{c}	0.11^{a}	0.12^{b}	0.006^{b}	0.005^{a}	0.010^{j}	0.006^{a}	0.33^{i}	0.003^{h}	0.0002^{a}	0.018^{d}
T_9	50%	0	0	1.60^{c}	0.12^{a}	0.12^{b}	0.006^{b}	0.005^{a}	0.047^{c}	0.007^{a}	0.42^{d}	0.007^{d}	0.0002^{a}	0.151^{b}
T_{10}			2.5	1.88^{a}	0.14^{a}	0.15^{b}	0.007^{b}	0.005^{a}	0.019^{f}	0.008^{a}	0.44^{b}	0.007^{d}	0.0002^{a}	0.032^{c}
T_{11}		750	0	1.45^{c}	0.11^{a}	0.12^{b}	0.004^{c}	0.005^{a}	0.015^{h}	0.007^{a}	0.31^{k}	0.019^{a}	0.0006^{a}	0.021^{d}
T_{12}			2.5	1.21^{d}	0.10^{a}	0.08^{c}	0.008^{b}	0.007^{a}	0.010^{j}	0.007^{a}	0.28^{l}	0.004^{g}	0.0001^{a}	0.020^{d}
T_{13}	75%	0	0	1.38^{c}	0.10^{a}	0.13^{b}	0.039^{a}	0.005^{a}	0.009^{k}	0.006^{a}	0.39^{e}	0.003^{h}	0.0002^{a}	0.018^{d}
T_{14}			2.5	1.59^{c}	0.11^{a}	0.12^{b}	0.006^{b}	0.004^{a}	0.013^{i}	0.006^{a}	0.45^{a}	0.009^{c}	0.0002^{a}	0.176^{a}
T_{15}		750	0	1.91^{a}	0.12^{a}	0.15^{b}	0.006^{b}	0.006^{a}	0.017^{g}	0.007^{a}	0.45^{a}	0.006^{e}	0.0003^{a}	0.035^{c}
T_{16}			2.5	1.44^{c}	0.13^{a}	0.12^{b}	0.003^{c}	0.005^{a}	0.012^{i}	0.008^{a}	0.35^{h}	0.007^{d}	0.0003^{a}	0.021^{d}
Pool standard error (PSE)				0.07	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01

At *P*<0.05, means within columns with distinct superscripts differ significantly. Data are the means of three replicas.

Table IV. Effect of canola meal-based diets supplemented with phytase and citric acid on the Apparent digestibility coefficients of minerals by *C. carpio* fingerlings.

Test diets	Fish meal replacement levels (%)	Phytase (FTU kg^{-1})	Citric acid (%)	Ca	Na	K	Fe	Cu	Zn	Mn	P	Mg	Al	Cr
T_1	0%	0	0	51.50^{d}	33.17^{f}	42.95^{g}	53.86^{b}	43.15^{e}	51.46^{d}	32.97^{j}	34.65^{g}	44.65^{e}	36.41^{d}	32.14^{f}
T_2			2.5	58.79^{b}	50.56^{b}	49.43^{d}	52.14^{b}	49.85^{c}	60.29^{b}	41.87^{h}	43.08^{e}	46.41^{d}	46.50^{b}	37.55^{e}
T_3		750	0	57.94^{c}	44.00^{d}	45.79^{f}	49.52^{c}	45.38^{d}	62.62^{a}	44.92^{g}	44.36^{e}	41.68^{f}	35.04^{d}	42.71^{c}
T_4			2.5	49.88^{d}	42.88^{d}	43.45^{g}	45.37^{d}	43.23^{e}	52.08^{d}	36.80^{i}	40.74^{f}	38.96^{g}	33.93^{d}	35.88^{f}
T_5	25%	0	0	40.56^{f}	42.69^{d}	39.80^{h}	35.75^{i}	38.79^{e}	41.00^{f}	31.40^{j}	37.13^{f}	37.66^{g}	32.97^{d}	33.07^{f}
T_6			2.5	49.83^{d}	43.17^{d}	42.62^{g}	49.19^{c}	41.14^{e}	51.46^{d}	41.97^{h}	40.65^{f}	44.62^{e}	36.08^{d}	34.81^{f}
T_7		750	0	57.45^{c}	50.56^{b}	51.76^{c}	43.48^{e}	47.18^{d}	58.29^{b}	47.86^{f}	45.68^{e}	45.61^{d}	42.50^{c}	45.55^{b}
T_8			2.5	58.94^{b}	49.33^{b}	51.46^{c}	46.85^{d}	39.05^{e}	60.96^{b}	50.25^{e}	44.99^{e}	41.21^{f}	38.38^{d}	38.71^{e}
T_9	50%	0	0	49.54^{d}	46.88^{c}	45.12^{f}	42.70^{e}	37.24^{e}	49.74^{e}	45.80^{g}	38.81^{f}	41.60^{f}	33.26^{d}	43.88^{c}
T_{10}			2.5	45.56^{e}	41.69^{d}	41.46^{g}	39.42^{g}	33.79^{f}	42.67^{f}	50.40^{e}	39.73^{f}	37.36^{g}	36.64^{d}	40.07^{d}
T_{11}		750	0	50.16^{d}	38.84^{e}	48.95^{d}	45.86^{d}	41.13^{e}	55.46^{c}	56.97^{b}	59.60^{b}	54.58^{b}	48.41^{b}	38.81^{e}
T_{12}			2.5	63.12^{a}	60.56^{a}	70.76^{a}	73.14^{a}	73.18^{a}	63.29^{a}	67.19^{a}	70.78^{a}	68.07^{a}	59.84^{a}	52.69^{a}
T_{13}	75%	0	0	58.61^{b}	44.33^{d}	55.13^{b}	40.52^{f}	46.05^{d}	59.29^{b}	48.91^{f}	48.45^{d}	41.67^{f}	33.04^{d}	38.38^{e}
T_{14}			2.5	45.88^{c}	41.21^{d}	54.45^{b}	45.37^{d}	40.57^{e}	52.74^{d}	50.47^{e}	38.91^{f}	39.26^{g}	28.93^{e}	35.67^{f}
T_{15}		750	0	40.22^{f}	50.35^{b}	48.46^{d}	38.75^{h}	36.48^{e}	51.67^{d}	54.06^{c}	40.13^{f}	38.18^{g}	26.64^{e}	38.85^{e}
T_{16}			2.5	49.50^{d}	59.17^{a}	47.28^{e}	49.86^{c}	60.39^{b}	48.46^{e}	52.63^{d}	54.32^{c}	50.23^{c}	39.74^{d}	34.88^{f}
Pool standard error (PSE)				1.84	1.43	1.57	2.13	2.10	1.64	1.52	1.40	1.82	2.28	1.82

At $P<0.05$, means within columns with distinct superscripts differ significantly. Data are the means of three replicas.

when compared to control (without any supplementation) and other experimental diets. Thus, supplementation with 2.5% CA and 750 FTU kg^{-1} PHY in 50% CM based diet was optimum to improve the fish performance in terms of minerals digestibility. The T_{11} diet (750 FTU kg^{-1} PHY in 50% CM based diet) also showed the second-lowest mineral excretion in the feces, representing high digestibility of minerals.

DISCUSSION

Fishmeal is a common protein source from animals in the aquaculture feeding because of its good amino acid content, higher palatability, and enhanced digestibility. However, the usage of fishmeal in aquatic diets increases costs of production as stocks of wild fish are depleting. As a result, there is a pressing requirement to look for novel and less expensive alternate sources of protein (Mugwanya *et al*., 2023). Currently, plant proteins are increasingly being used as fishmeal alternatives in aquatic diets because of their higher nutritional value, widespread availability, and lower cost. CM, recognized for its superior mineral content, has emerged as a plant protein source of choice to substitute fishmeal in fish diets. Its utilization provides a promising solution for maintaining adequate mineral resources in fish feed formulations. However, an anti-nutritional factor phytate is present in plants that hamper the availability and absorption of minerals in fish, by forming complexes with minerals, limiting their bioavailability (Selamoglu *et al*., 2015; Rizwanuddin *et al*., 2023). In the current study, CM based diets having identical mineral compositions were fed to *C. carpio*. However, CA and PHY were supplemented in these diets to improve the mineral digestibility in *C. carpio* fingerlings.

According to current findings, the 50% substitution of CM for fishmeal with 2.5% CA and 750 FTU kg^{-1} PHY supplements emerged as the most optimal combination that reacted synergistically to reduce the excretion of essential minerals into the aquatic environment through feces and improve the minerals bioavailability. Consistent with the findings of the present study, Shah *et al*. (2021) also observed a reduction in mineral excretion (P, K, Mg, Na, Mn, Fe, and Cu) when *Labeo rohita* fingerlings were fed a soybean meal-based diet combined with 3% CA and 1000 FTU/kg PHY. This indicates that the combined supplementation of CA and PHY synergistically decreases mineral excretion in fish feces and enhances mineral digestibility. Similarly, Afzal *et al*. (2020) discovered significant improvements in the digestibility of minerals, i.e., Ca, Cu, Fe, P, Na, and K in *L. rohita* given a sunflower meal-based diets with 2% CA and 1000 FTU/kg PHY. In another study by Afzal *et al*. (2019), the digestibility of Ca, Cu, Fe, K, Mg, Mn, P, and Zn in *L. rohita* juveniles was significantly enhanced ($P<0.05$) fed with soybean meal based diet combined with 2% CA and 1000 FTU/kg PHY compared to the control diet. Similarly, Hussain *et al*. (2018) reported the highest apparent digestibility coefficients (ADC%) of minerals (Na: 64%, Fe: 64%, Cu: 68%, Zn: 74%, Ca: 68%, K: 62%, Mg: 53%, P: 77%, and Mn: 67%) in *Cirrhinus mrigala* fingerlings fed a corn gluten meal-based diet with CA 5% and PHY 500 FTU/kg. They suggested that the use of CA and PHY increased the availability of minerals for fish by releasing chelated minerals, resulting in reduced excretion of essential minerals into the aquatic environment and, consequently, mitigating aquatic pollution. Several studies have consistently demonstrated that the synergistic supplementation of PHY and CA in diets based on plant proteins enhances the availability of minerals for fish. Examples include diets based on canola meal with 3% CA and 1000 FTU/kg PHY for *L. rohita* fingerlings (Habib *et al*., 2018), sunflower meal with 2% CA and 1000 FTU/kg PHY for *L. rohita* juveniles (Rabia *et al*., 2017), cottonseed meal with 4% CA and 400 FTU/kg PHY for *L. rohita* (Hussain *et al*., 2016), and soybean meal with 3% CA and 1000 FTU/kg PHY for *L. rohita* (Shah *et al*., 2016; Baruah *et al*., 2007).

In present study, it was observed that supplementation of CM with CA and PHY resulted in the maximum digestibility of various minerals, including Ca, K, Fe, Cu, Mn, P, Mg, and Al, in *C. carpio* fingerlings. This improved mineral digestibility proved the effective phytic acid hydrolysis by PHY. The addition of CA enhanced digestion and lowered the pH of the gastrointestinal tract, providing optimal conditions for PHY activity. As a result, the minerals became more bioavailable to *C. carpio* fingerlings, leading to improved growth performance. Furthermore, the synergistic incorporation of CA and PHY resulted in the minimal excretion of essential minerals into the aquatic ecosystem compared to the control diet without any supplementation. Similar findings have been reported by several researchers in *L. rohita* (Baruah *et al*., 2007; Shah *et al*., 2016, 2021; Rabia *et al*., 2017; Habib *et al*., 2018; Afzal *et al*., 2019, 2020) and *C. mrigala* (Hussain *et al*., 2018). In conclusion, the CA and PHY addition facilitated the dissociation of phytate chelated structure, leading to the release and assimilation of essential minerals in fish. This resulted in improved mineral digestibility and reduced excretion of essential minerals through feces. The findings of this study aligned with previous research demonstrating that the supplementation of plant-based diets with CA enhances mineral absorption in fish (Sugiura *et al*., 2001).

The current findings revealed a positive correlation between CA and PHY, highlighting their synergistic impact on mineral absorption. It is plausible that CA played a crucial role in optimizing the pH levels within the fish's gastrointestinal tract, facilitating the efficient functioning of PHY and thereby maximizing the mineral digestion process. Furthermore, demonstrated that diet acidification led to a reduction in the rate of stomach emptying, allowing for an extended time frame for PHY to exert its effects. Notably, prior research conducted by Baruah *et al*. (2007) and Sugiura *et al*. (2001) has also documented enhanced mineral absorption in response to the incorporation of CA and PHY in plant-based diets, further validating the beneficial impacts of these supplements.

The current study revealed that the digestibility of minerals was significantly lower in the group fed the control diet (without CA or PHY) compared to the groups fed diets having CA or PHY or both. Similarly, Zhang *et al*. (2016) found improvement in minerals bioavailability in Large yellow croaker (*Larimichthys crocea)* fed citric acid treated soybean meal-based diets. Notably, the improved digestibility of phosphorus (P) was found to have a positive impact on the digestibility of other minerals, including Ca, K, Fe, Cu, Mn, Mg, and Al. This finding aligns with previous research highlighting the beneficial role of PHY supplementation in releasing chelated phosphorus and enhancing mineral availability in various fishes (Baruah *et al*., 2007; Cao *et al*., 2007; Dalsgaard *et al*., 2009; Pontes *et al*., 2021; Hussain *et al*., 2022). In a study by Nwanna and Schwarz (2007), it was discovered that fish groups supplemented with PHY exhibited higher P digestibility and reduced P discharge compared to the control group. The incorporation of PHY in plant-based diets has shown promising results in producing less expensive and environmentally friendly aqua-feeds by enhancing nutrients digestibility and minimizing nutrient discharge into the aquatic environment (Priya *et al*., 2023). Therefore, the supplementation of PHY in plant meal-based diets is expected to contribute to the reduction of aquatic pollution.

CONCLUSION

In conclusion, the findings of this study showed that the T_{12} diet, which involved a 50% replacement level of CM supplemented with CA and PHY, demonstrated a significant enhancement in mineral digestibility for *C. carpio* fingerlings. This finding underscores the potential of incorporating plant by-products like CM as a viable and sustainable alternative to fishmeal in the development of environment friendly and cost-effective aqua feed for *C. carpio* fingerlings. Additionally, the PHY and CA supplements in plant-based diets maximize their effectiveness by reducing the anti-nutritional components and boosting mineral bioavailability.

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Ethical statement

All applicable institutional, national and international guidelines for the care and use of animals were followed.

Statement of conflict of interest

The authors have declared no conflict of interest.

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