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SYNERGISTIC EFFECTS OF CITRIC ACID AND PHYTASE SUPPLEMENTED CANOLA MEAL BASED DIET ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY OF *CYPRINUS CARPIO* FINGERLINGS

M. Z. Arsalan[1,2], S. M. Hussain[2*], A. Javid[3], M. Hussain[4], R. Manzoor[5], M. M. Jalees[6], M. M. Rehan[7] and A. Sharif[2]

[1]Department of Life Sciences, Faculty of Natural and Applied Sciences, Khwaja Fareed University of Engineering & Information Technology, Rahim Yar Khan, Pakistan
[2]Fish Nutrition Lab, Department of Zoology, Government College University, Faisalabad, Pakistan
[3]Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[4]Department of Fisheries and Aquaculture, University of Okara, Okara, Pakistan
[5]Freshwater Aquaculture Collaborative Innovation Center of Hubei Province, Hubei Provincial Engineering Laboratory for Pond Aquaculture, College of Fisheries, Huazhong Agricultural University, Wuhan,430070, China
[6]Department of Microbiology, Cholistan University of Veterinary and Animal Sciences Bahawalpur
[7]Southeast University Nanjing, China
[*]Corresponding author's Email drmakhdoomhussain@gcuf.edu.pk

ABSTRACT

A 3×3 factorial arrangement was used to determine the synergistic effects of citric acid (CA) and phytase (PHY) supplemented canola meal (CM) based diet on growth performance and nutrient digestibility of *Cyprinus carpio* fingerlings. Canola meal was used as a main ingredient to prepare diets by replacing fishmeal at graded levels such as: 0%, 25%, 50% and 75%. These diets were further supplemented by different levels of CA (0% and 2.5%) and PHY (0 and 750 FTU kg^{-1}) to formulate a total of 16 test diets. Completely Randomized Design (CRD) was followed to add diets to the fish groups. Chromic oxide (Cr_2O_3) was added as an indigestible marker. Fish were fed at the rate of 5% of their live wet weight. The findings of study revealed that the maximum weight gain% (267.81%), SGR (1.44) and the lowest value of FCR (1.32) were noted when fingerlings were fed on test diet T_{12} containing 50% canola meal with 750 FTUkg^{-1} PHY and 2.5% CA supplementation as compared to fish fed on control diet (T_1) having 0% canola meal and without any concentration of CA and PHY. Similarly, optimum nutrient digestibility values such as crude protein (75.06%), crude fat (83.31%) and gross energy (66.45%) were noted when fish fed on the same diet (T_{12}). It may be concluded that fish meal could be optimally replaced with canola meal up to 50%, when supplemented with PHY (750 FTU kg^{-1}) and CA (2.5%).

Key words: *C. carpio*, phytase, citric acid, canola meal, growth, nutrient digestibility

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INTRODUCTION

Common carp (*Cyprinus carpio*) being the most commonly cultivated teleost species is the major food source in more than a hundred countries around the world. Generally, the term "aquaculture products" is used for the aquatic animal and plant products that are produced by marine and freshwater fisheries (Selamoglu, 2021). Feed, a significant component of aquaculture production, is a major impediment to the aquaculture sector's development and expansion (Gabriel *et al.,* 2007). Fishmeal is the most common ingredient in aqua-feeds because of its higher palatability and nutritional value (NRC, 2011). In fish feed, replacement of fishmeal, because of its rising cost and unpredictable supply, with cost effective and easily available plant or animal-based proteins/substances is necessary (Mahboob *et al.,* 2014). Canola meal being a protein-rich (38%) and easily digestible feed is a suitable substitute for fish meal. Apart from having best amino acid balance, it is very good source of vitamins, especially vitamin B and minerals like selenium, Sulphur, and phosphorus among other commercially available plant protein sources (Enami, 2011; Canola Council of Canada, 2019). Furthermore, higher total content of minerals in canola meal leads to its higher availability for animals, which is even better than that of soybean meal (Ghazalah *et al.,* 2022). When compared to soybean meal and fish meal, canola meal is reported to be more cost effective (Hussain *et al.,* 2017).

In fish feed, inclusion of plant-based proteins causes major problems because of the presence of anti-nutritional factors i.e. phytic acid. Phytic acid, the primary source of phosphorus in plants accounts for 50-80% of total phosphorus (Ravindran and Kornegay, 1993). It reduces the digestion of nutrients that are important for fish health, such as proteins (Liu *et al.,*

2013) resulting in biological unavailability of these substances and growth suppression (Shah *et al.,* 2021). Phytase, also known as myo-inositol hexaphosphate phosphohydrolase, can hydrolyze phytic acid-bound nutrients and make them available to fish (Gatlin III *et al*., 2007). The addition of phytase to fish feed has resulted in improved chelated phosphorus absorption in Nile tilapia and rainbow trout (Verlhac-Trichet *et al*., 2014). Organic acid has been shown to be an effective antibacterial agent that improves disease resistance, growth and nutrient utilization (NRC, 2011). Simons *et al.* (1990) found that phytase performed best at pH levels of 2.5 and 5.0-5.5. The addition of citric acid (CA) lowers the gastrointestinal pH in fish to the optimum range for phytase action. As a result, it is believed to be a good way to boost phytase activity (Nourmohammadi *et al.,* 2011).

Thus, the major aim of this study was to examine the synergistic effects of citric acid and phytase, as well as their interaction on the growth performance and nutrient digestibility of *C. carpio* fingerlings fed canola meal-based diet.

MATERIALS AND METHODS

This research work was conducted in the Fish Nutrition Laboratory at Department of Zoology, Government College University, Faisalabad from June to September, 2021.

Fish and Experimental Conditions: *C. carpio* fingerlings were purchased from the Government Fish Seed Hatchery, Satiana Road, Faisalabad. The fingerlings were brought in Fish Nutrition Laboratory and stocked in V-shaped fish tanks for acclimatization. These V-shaped fish tanks were specially designed for the collection of fecal material. Fingerlings were acclimatized for 15 days in the laboratory conditions. Fish were fed on the basal diet twice a day during this acclimatization period, used in subsequent digestibility studies (Allan and Rowland, 1992). Dissolved oxygen was provided 24 hours a day through a capillary system. Before beginning of the feeding trial, all fingerlings were treated with NaCl solution having concentration 5gL^{-1} to remove ecto-parasites as well as to prevent fungal infection.

Experimental Design: The experimental diets were made with canola meal as the test ingredient. Fish meal (FM) was replaced with canola meal (CM) at various levels such as 0%, 25%, 50%, and 75%. These four replacement-based diets were further supplemented with citric acid (0% and 2.5%) and phytase (0 and 750 FTU kg^{-1}) to formulate 16 test diets named as T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9, T_{10}, T_{11}, T_{12}, T_{13}, T_{14}, T_{15} and T_{16} (Table 1). However, the concentration of rice polish (3%), fish oil (6%), vitamins premix (1%), minerals premix (1%) and chromic oxide (1%) was same in all test diets. These test diets were fed to sixteen different groups of fish in the experimental tanks using completely randomized design (CRD). Each treatment had three replicates, each containing 15 fingerlings. The experiment lasted for 90 days. CM-based CA and PHY supplemented diets were compared to determine growth and nutrient digestibility by using a completely randomized design.

Feed Ingredients and Experimental Diets: The ingredients for the feed (Table 1) were purchased from a local market and before the formulation of the experimental diets they were analyzed for chemical composition according to AOAC (1995). The feed ingredients were finely grinded at sieve level of 0.5 mm. All of the feed ingredients were mixed for 10 minutes in an electric mixer, and the fish oil was gradually added while the ingredients were being mixed. To make suitable dough, 10% water was added, and the feed pellets (3mm) of test diets were made by using the pelleting machine. Distilled water (25 mL) was used for making the appropriate concentrations of 0 and 750 FTU kg^{-1} of phytase that was sprayed on test diets (Robinson *et al.,* 2002). The control diet (0 FTU kg^{-1}) was sprayed with a similar volume of distilled water to maintain a similar amount of moisture. Before use, all of the prepared diets were dried and stored at 4°C.

Feeding Protocol and Sample Collection: Fish were fed twice a day throughout the feeding trial. At the beginning of the experiment, they were fed at a rate of 5% of their live wet weight on their prescribed diet, and then adjusted to a daily feed intake. Each test diet had three replicates, with 15 fingerlings (average weight: 5.75 g fish^{-1}) stocked in it. Each tank's uneaten diet was removed after a two-hour feeding time. To remove the particles of uneaten diets, the tanks were completely washed before being filled with water. Feces were collected from each tank's fecal collection tube after two hours. The feces from each treatment were dried in an oven and then stored for chemical analysis.

Chemical Analysis of Feed and Feces: Feed materials, experimental diets, and feces samples were homogenized with a mortar and pestle before being analyzed by standard methods (AOAC, 1995). Moisture was determined by oven drying at 105°C for 12 hours; crude protein (N×6.25) by micro-Kjeldahl apparatus; crude fat by petroleum ether extraction method through Soxtec HT2 1045 system; crude fiber was determined by loss on ignition of dried lipid-free residues after digestion with 1.25% H_2SO_4 and 1.25% NaOH; ash was determined by ignition at 650°C for 12 hours in an electric furnace (Eyela-TMF). Oxygen bomb calorimeter was used to determine gross energy in feed and feces samples.

Chromic Oxide Estimation: After oxidation with molybdate reagent, chromic oxide contents in diets and feces were determined by using a UV-VIS 2001 spectrophotometer (Divakaran *et al.,* 2002).

Growth Study: During the experiment, fish in each tank were bulk weighed every 15th day to examine growth performance of *C. carpio* fingerlings. Standard formulae were used to calculate the weight gain (%), feed conversion ratio (FCR) and specific growth rate (SGR) of fingerlings.

$$Weight\ gain\ \% = \frac{(Final\ weight - Initial\ weight)}{Initial\ weight} \times 100$$

$$FCR = \frac{Total\ dry\ feed\ intake\ (g)}{Wet\ weight\ gain\ (g)}$$

$$SGR = \frac{Final\ Weight\ (g) - Initial\ Weight(g)}{No\ of\ trial\ days} \times 100$$

Calculation of Digestibility: Cr_2O_3 was added in the diet as an indigestible marker and the acid digestion method was used to determine its amount in both feed and feces (Divakaran *et al.,* 2002). The molybdate reagent was used for oxidation, and the UV-VIS 2001 Spectrophotometer was set at 370 nm absorbance level. The values obtained from feces and feed analysis were used to conduct digestibility studies in term of apparent nutrient digestibility coefficients (ADC %) following the formula used by Arsalan *et al.* (2021)

$$ADC\ (\%) = 100 - 100 \times \frac{\%\ marker\ in\ diet \times \%\ nutrient\ in\ feces}{\%\ marker\ in\ feces \times \%\ nutrient\ in\ diet}$$

Statistical Analysis: The results were statistically analyzed by using the CoStat Computer Package (Version 6.303, PMB 320, Monterey, CA, 93940 USA) and three-way analysis of variance (ANOVA) (Steel *et al*., 1996). Data was expressed as mean with standard deviations. Significance level was established at $p \leq 0.05$. When significant differences between diets were determined, Tukey's Honesty Significant Difference Test was used to compare means (Snedecor and Cochran, 1991).

RESULTS

According to the present findings, CA and PHY supplementation synergistically enhanced nutrient digestibility in fish, resulting in improved growth performance. There was no difference in initial weight of fingerlings and mean initial weight was 5.73±0.015g. Maximum improved weight gain, weight gain (%) as well as best FCR, SGR, survival rate and nutrient digestibility were observed when *C. carpio* fingerlings fed on CA and PHY supplemented CM based diets as compared to control diet. The maximum weight gain (15.33g), weight gain % (267.81 %), best FCR value (1.32), highest SGR (1.44) and survival rate (100%) of *C. carpio* fingerlings were noted in fish group fed T_{12} test diet which have 50% replacement of CM based diet supplemented with 2.5% CA and 750 FTU kg^{-1} PHY level. The second highest value of weight gain (15.11 g), weight gain % (263.96%), FCR value (1.34), SGR (1.43) and survival rate (100%) were observed when fingerlings were fed on T_{11} test diet which have 50% replacement of CM based diet supplemented with 0% CA and 750 FTU kg^{-1} PHY level. Table 2 shows that these values were significantly ($p \leq 0.05$) different from the control group (weight gain 9.48g, weight gain% 165.31%, FCR 2.11, SGR 1.08, and survival rate 97%).

The percentage of nutrients (crude protein, crude fat and gross energy) in feces and nutrient digestibility are given in tables 3, 4 and 5. Results revealed that there was a significant difference in nutrients discharge through feces when *C. carpio* fingerlings were fed on PHY and CA supplemented CM based diet. *C. carpio* fingerlings fed test diet T_{12} showed maximum digestibility values of crude protein (75.06%), crude fat (83.31%), and gross energy (66.45%). These maximum values were found to be significantly *($p \leq 0.05$)* different from all other diet groups. However, the minimum digestibility values for crude protein (50.77%), crude fat (63.95%), and gross energy (51.57%) were observed when fingerlings fed test diet T_1. Due to increased nutrient digestibility, growth performance of fish fed diet T_{12} was also enhanced as shown in Table 2.

Table 1 Ingredients composition (%) of test diets.

Test diets	Fishmeal Protein Replacement Levels (%)	Fishmeal	Canola meal	PHY (FTU kg^{-1})	CA (%)	Wheat flour
T_1	0%	48	18	0	0	22
T_2		48	18		2.5	19.5
T_3		48	18	750	0	22
T_4		48	18		2.5	19.5
T_5	25%	36	35	0	0	17
T_6		36	35		2.5	14.5
T_7		36	35	750	0	17
T_8		36	35		2.5	14.5

T_9	50%	24	53	0	0	11
T_{10}		24	53		2.5	8.5
T_{11}		24	53	750	0	11
T_{12}		24	53		2.5	8.5
T_{13}	75%	12	71	0	0	5
T_{14}		12	71		2.5	2.5
T_{15}		12	71	750	0	5
T_{16}		12	71		2.5	2.5

[*]Each 100Kg of Vit premix contains

Vit A	2000,000IU	Vit B_6	600 mg
Vit D	400,000 IU	Vit B_{12}	2,000 mg
Vit E	4000 mg	Vit C	2,000 mg
Vit K_3	900 mg	Folic acid	200 mg
Vit B_1	200 mg	Calcium pantothenate	2,000 mg
Vit B_2	3000 mg	Nicotinic acid	10,000 mg

[**]Each Kg mineral premix contains

Na, 45g;	Cu, 600mg;	I, 40mg
Ca, 155g;	Mg, 55g;	Co, 40mg
Zn, 3000 mg;	P, 135g;	Se, 3mg
Fe, 1000 mg;	Mn, 2000mg;	

Table 2. Growth parameters of *C. carpio* fingerlings fed on canola meal based test diets supplemented with CA and PHY

Test diets	Fishmeal Protein Replacement Levels (%)	PHY	CA	Weight gain (g)	Weight gain (%)	FCR	SGR	Survival %
T_1	0%	0	0	9.48±0.020^m	165.31±0.68^m	2.11±0.03^a	1.08±0.002^l	97
T_2			2.5	11.67±0.036^k	203.43±1.17^k	1.50±0.02^d	1.23±0.004^j	97
T_3		750	0	11.97±0.049^j	208.65±1.48^j	1.62±0.02^c	1.25±0.005^i	98
T_4			2.5	13.25±0.062^g	230.43±0.38^g	1.51±0.01^d	1.32±0.001^g	98
T_5	25%	0	0	10.94±0.085^l	190.48±2.30^i	1.81±0.03^b	1.18±0.008^k	96
T_6			2.5	12.92±0.032^h	225.72±1.10^i	1.57±0.00^d	1.31±0.003^g	98
T_7		750	0	13.15±0.041^g	229.34±1.12^g	1.54±0.02^d	1.32±0.003^g	96
T_8			2.5	14.22±0.077^d	247.64±0.88^d	1.42±0.02^f	1.38±0.002^d	100
T_9	50%	0	0	13.14±0.335^g	228.58±6.15^g	1.54±0.05^d	1.32±0.020^g	98
T_{10}			2.5	12.43±0.063^i	216.61±1.79^i	1.63±0.01^c	1.28±0.006^h	97
T_{11}		750	0	15.11±0.045^b	263.96±0.12^b	1.34±0.02^g	1.43±0.000^b	100
T_{12}			2.5	15.33±0.051^a	267.81±1.57^a	1.32±0.01^g	1.44±0.004^a	100
T_{13}	75%	0	0	12.29±0.036^i	214.23±0.26^i	1.65±0.02^c	1.27±0.000^h	98
T_{14}			2.5	13.47±0.045^f	234.53±1.34^f	1.50±0.02^d	1.34±0.004^f	100
T_{15}		750	0	13.85±0.098^e	241.68±1.16^e	1.46±0.02^e	1.36±0.003^e	100
T_{16}			2.5	14.57±0.037^c	254.04±1.21^c	1.39±0.01^f	1.40±0.003^c	100

Means within columns having different superscripts are significantly different at $p \leq 0.05$.
Data are means of three replicates.

Table 3. Analyzed composition (%) of nutrients in diets of *C. carpio* fingerlings fed on PHY and CA supplemented canola meal based test diets.

Test Diets	Fishmeal Protein Replacement Levels (%)	PHY (FTU kg^{-1})	CA (%)	Crude protein	Crude fat	Gross energy
T_1	0%	0	0	30.54±0.066	8.05±0.020	3.41±0.020
T_2			2.5	30.62±0.090	8.08±0.040	3.43±0.030
T_3		750	0	30.58±0.035	8.05±0.030	3.40±0.017
T_4			2.5	30.65±0.079	8.05±0.030	3.42±0.025

T_5	25%	0	0	30.64±0.050	8.07±0.020	3.41±0.036
T_6			2.5	30.63±0.090	8.04±0.020	3.43±0.017
T_7		750	0	30.66±0.049	8.08±0.015	3.41±0.025
T_8			2.5	30.66±0.040	8.07±0.040	3.42±0.026
T_9	50%	0	0	30.65±0.077	8.05±0.030	3.42±0.030
T_{10}			2.5	30.66±0.062	8.03±0.015	3.42±0.020
T_{11}		750	0	30.62±0.03	8.04±0.010	3.41±0.020
T_{12}			2.5	30.67±0.060	8.06±0.010	3.41±0.015
T_{13}	75%	0	0	30.68±0.045	8.05±0.020	3.41±0.010
T_{14}			2.5	30.65±0.080	8.08±0.040	3.43±0.020
T_{15}		750	0	30.69±0.020	8.04±0.010	3.42±0.025
T_{16}			2.5	30.63±0.041	8.03±0.010	3.42±0.040

Means within columns having different superscripts are significantly different at $p \leq 0.05$
Data are means of three replicates

Table 4. Analyzed composition (%) of nutrients in feces of *C. carpio* fingerlings fed on PHY and CA supplemented canola meal based test diets.

Test Diets	Fishmeal Protein Replacement Levels (%)	PHY (FTU kg^{-1})	CA	Crude protein	Crude fat	Gross energy
T_1	0%	0	0	16.00±0.44^{a}	2.32±0.03^{a}	1.75±0.01^{a}
T_2			2.5	14.25±0.03^{c}	1.75±0.03^{d}	1.70±0.03^{b}
T_3		750	0	13.76±0.06^{d}	1.99±0.02^{c}	1.64±0.04^{c}
T_4			2.5	11.00±0.03^{j}	1.48±0.02^{d}	1.49±0.01^{h}
T_5	25%	0	0	15.21±0.03^{b}	2.18±0.02^{a}	1.71±0.03^{b}
T_6			2.5	12.03±0.07^{h}	1.60±0.01^{d}	1.54±0.02^{f}
T_7		750	0	11.63±0.04^{i}	1.54±0.01^{d}	1.50±0.02^{g}
T_8			2.5	9.86±0.03^{m}	1.75±0.03^{d}	1.33±0.02^{k}
T_9	50%	0	0	13.10±0.02^{e}	1.83±0.02^{d}	1.64±0.02^{c}
T_{10}			2.5	12.15±0.06^{g}	1.67±0.03^{d}	1.58±0.02^{e}
T_{11}		750	0	8.90±0.01^{o}	1.16±0.01^{g}	1.25±0.01^{m}
T_{12}			2.5	8.48±0.03^{p}	1.12±0.01^{g}	1.23±0.02^{n}
T_{13}	75%	0	0	12.69±0.07^{f}	1.71±0.02^{d}	1.63±0.02^{d}
T_{14}			2.5	10.8±0.04^{k}	1.75±0.03^{d}	1.45±0.01^{i}
T_{15}		750	0	10.38±0.04^{l}	1.38±0.04^{e}	1.39±0.01^{j}
T_{16}			2.5	9.29±0.02^{n}	1.23±0.02^{f}	1.29±0.01^{l}

Means within columns having different superscripts are significantly different at $p \leq 0.05$
Data are means of three replicates

Table 5 Apparent digestibility coefficients (ADC%) of nutrients for *C. carpio* fingerlings fed on PHY and CA supplemented canola meal based test diets.

Test Diets	Fishmeal Protein Replacement Levels (%)l	PHY (FTU kg^{-1})	CA	Crude protein	Crude fat	Gross energy
T_1	0%	0	0	50.77±0.78^{o}	63.95±0.55^{i}	51.57±0.58^{k}
T_2			2.5	54.40±1.97^{m}	49.29±0.44^{j}	53.11±1.30^{i}
T_3		750	0	57.99±0.41^{l}	69.23±0.23^{g}	54.77±1.95^{h}
T_4			2.5	66.05±0.59^{g}	76.80±0.28^{c}	58.66±1.02^{f}
T_5	25%	0	0	53.05±0.53^{n}	65.93±0.39^{h}	52.50±0.66^{j}
T_6			2.5	63.40±0.89^{i}	75.21±0.42^{d}	58.18±1.91^{f}
T_7		750	0	64.34±1.14^{h}	76.12±0.39^{c}	58.61±1.42^{f}
T_8			2.5	70.55±0.51^{d}	79.65±0.08^{b}	64.33±0.78^{c}
T_9	50%	0	0	60.66±1.22^{k}	72.06±0.63^{f}	55.82±2.22^{h}
T_{10}			2.5	63.24±0.19^{i}	74.00±0.45^{d}	56.98±0.61^{g}
T_{11}		750	0	73.79±0.40^{b}	82.57±0.23^{a}	66.79±0.66^{a}

T_{12}			2.5	75.06±0.12^{a}	83.31±0.26^{a}	66.45±0.84^{a}
T_{13}	75%	0	0	61.35±0.70^{j}	73.57±0.70^{e}	55.23±0.71^{h}
T_{14}			2.5	67.39±0.65^{f}	78.28±0.14^{b}	61.75±1.07^{e}
T_{15}		750	0	68.67±0.09^{e}	78.82±0.47^{b}	62.21±0.46^{d}
T_{16}			2.5	72.41±0.35^{c}	81.37±0.24^{a}	65.60±0.28^{b}

Means within columns having different superscripts are significantly different at $p \leq 0.05$

Data are means of three replicates

DISCUSSION

In the present study, different levels of CA (0% and 2.5%) and PHY (0, 750 FTU kg^{-1}) were used in CM based test diets to determine their effects on morphometric parameters of *C. carpio* fingerlings. The results of mean weight gain revealed that all formulated diets were nutritionally adequate. Studies of Arsalan *et al.* (2021) and Ahmad *et al.* (2021) with *C. mrigala* and *L. rohita* fingerlings, respectively revealed the similar results of improved growth performance when 50% FM was replaced with CM and cottonseed meal, respectively, and supplemented with 2.5% CA and 750 FTU kg^{-1} level of PHY. This replacement level of fish meal by plant meals along with CA and PHY supplementation proved beneficial in improving morphometric parameters of fish. In another study, acidified and PHY treated corn gluten (30%) meal-based diet was fed to *C. mrigala* fingerlings that resulted in increased growth performance at 5% CA and 500 FTU kg^{-1} level of PHY supplementation (Hussain *et al.,* 2015). Similar to this study, the best FCR value (1.03) was found in *C. mrigala* fingerlings fed PHY (1000 FTU kg^{-1}) and CA (2.5%) supplemented guar meal-based diets, which was significantly different ($p \leq 0.05$) from all other treatments (Hussain *et al.,* 2020). Literature proved that dietary acidification and PHY supplementation in plant meal-based diets had positive effects on growth performance of fish. This improvement may be due to the fact that CA and PHY added plant ingredients based diets improve the activity of digestive enzymes which ultimately resulted in improvement of fish growth. Similarly, CA and PHY inclusion in plant meal-based fish diet intensify PHY activity by increasing H^{+} ions release thus reducing stomach pH and causing break down of PHY complexes (Shah *et al.,* 2015; 2021).

In our study, the growth performance of *C. carpio* fingerlings was found maximum when fed 50% canola meal-based diet supplemented with CA (2.5%) and PHY (750 FTU kg^{-1}). However, in some other studies with *O. niloticus*, Mahmoud *et al.* (2019) and Nehad *et al.* (2020) reported significant ($p \leq 0.05$) improvement in growth performance (WG%: 67%, FCR: 1.50) and feed utilization when CA (30 g/Kg) was used in combination with PHY (1000 FTU/kg), respectively. Similarly, *L. rohita* fingerlings exhibited maximum growth performance when diet was supplemented with 3% CA and 500 (FTU kg^{-1}) PHY (Baruah *et al.,* 2007). Zhu *et al.* (2014) and Thiam *et al.* (2015) also reported that the interactive effects of CA and PHY proved to be beneficial in enhancing growth of yellow catfish and Nile tilapia fingerlings, respectively. In another feeding trial, *C. mrigala* fingerlings showed maximum improvement of growth parameters (WG% 401% and FCR 1.21) when fed different levels of CA and PHY (i.e. 5% and 500 FTU kg^{-1}) added corn gluten meal-based diet (Hussain *et al.,* 2015). Different studies suggested that for improving growth parameters, incorporation of PHY in fish feed should be ranged between 250-1500 FTUkg^{-1} levels (Cao *et al.,* 2007) while CA between 2.5-5% (Khajepour *et al.,* 2012).

In the light of experimental conditions tested here, it can be said that CA and PHY in CM based diets synergistically enhanced nutrient digestibility of *C. carpio* fingerlings, which resulted in improved growth performance. Thus, addition of PHY and CA is beneficial for the formulation of eco-friendly, nutritionally bioavailable and cost-effective feed for *C. carpio*. However, further investigation about different size and age group of fish is required.

Canola meal being a good source of essential nutrients, proteins and fat, also contains anti nutritional factors. Phytate is also present in all plant protein-based diets, and it can affect fish nutrient digestibility (Hussain *et al.,* 2015). Furthermore, it can bind with important amino acids in several fish species, reducing nutrient digestibility, particularly protein digestibility (Usmani and Jafri, 2002). In current study, *C. carpio* showed maximum digestibility of gross energy, crude protein and crude fat along with maximum retention but less excretion of nutrients when fed on test diet T_{12}. These results coincide with the findings of Arsalan *et al.* (2021). Similar results of improved nutrient digestibility and less nutrients discharged through feces were also found by Afzal *et al.* (2019). They stated that supplementation of PHY (1000 FTUkg^{-1}) and CA (2%) in SBM based diet significantly enhanced digestibility of *L. rohita* Juveniles compared to control diet. In another study with *L. rohita*, Shah *et al.* (2021) also found less nutrients discharge and highest nutrient availability of CP, EE and GE when fish fed with SBM based diet supplemented with 3% CA and 1000 FTUkg^{-1} level of PHY. Rabia *et al.* (2017) reported decreased amount of nutrients in feces and improved values of crude protein, crude fat and gross energy in *L. rohita* juveniles fed on sunflower meal-based diet supplemented with 2% CA and 1000 FTUkg^{-1} PHY. In contrast to the current findings, the interaction of CA and

PHY remained unsuccessful in improving digestibility of *L. rohita* and *C. carpio*, respectively (Baruah *et al.,* 2007; Phromkunthong *et al.,* 2010). However, Hussain *et al.* (2015) stated that when PHY (500 FTU/kg) and CA (5%) synergistically added to corn gluten meal (30%) based diets, nutrient digestibility of *C. mrigala* fingerlings was enhanced whereas other higher supplementation levels of PHY were unsuccessful in enhancing nutrient digestibility especially of crude protein. This variation in nutrient digestibility results may be due to differences in phytase sources, feed ingredients, stomach pH, feed processing and fish species (Liu *et al.,* 2013).

Conclusion: It is obvious from the present data that a significant difference existed in the nutrient digestibility of crude protein, crude fat and gross energy at both individual and interactive levels of CA and PHY supplemented CM based diets. When minimum amount of feces will be discharged into water, there will be low chances of eutrophication or water pollution. The findings also supported the hypothesis that addition of organic acid in the diet gives favorable environment to PHY for optimum performance. Thus, the dietary supplementation of both PHY and CA not only enhanced growth performance and nutrient digestibility, but it also increased nutrients retention in fish body but reduced nutrient excretion through feces in *C. carpio*.

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