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Original Article

Use of phytase and citric acid supplementation on growth performance and nutrient digestibility of *Cirrhinus mrigala* fingerlings fed on canola meal based diet

Uso de suplementação de fitase e ácido cítrico no desempenho do crescimento e digestibilidade de nutrientes de dedos *Cirrhinus mrigala* alimentados em dieta à base de refeição de canola

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Abstract

Fishmeal; being a limited and costly feed ingredient is continuously been substituted with locally available plant proteins. However, the occurrence of anti-nutritional factors in plant meal suppresses its potential to be fully replaced. Therefore, in this study we aimed to study the synergistic effects of dietary additives like citric acid and phytase enzyme supplementation on growth performance and nutrient digestibility of *Cirrhinus mrigala* fingerlings. Canola meal (CM) was used as a test ingredient to replace fishmeal (FM) as; 0%, 25%, 50% and 75%. These four diets were further supplemented by varying levels of phytase (0 and 750 FTU kg^{-1}) and citric acid (0% and 2.5%) to formulate total sixteen test diets 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}. Each treatment contained three replicates; applied to fish groups having 15 fingerlings each; following 3×3 factorial arrangement. 1% of chromic oxide was added as an inert marker. Maximum weight gain% (288%) and the lowest value of FCR (1.07) were recorded when fish was fed on diet T_{12} as compared to fish fed control diet (T_1). Similarly, optimum nutrient digestibility values such as crude protein (77%), crude fat (84%) and gross energy (70%) were noted on same level. It was concluded that 50% canola meal can optimally replace fishmeal when supplemented with phytase and citric acid at the levels of 750 FTU kg^{-1} and 2.5%, respectively.

Keywords: *C. mrigala*, growth, nutrient digestibility, fishmeal, canola meal.

Resumo

A farinha de peixe, por ser um ingrediente alimentar limitado e caro, é continuamente substituída por proteínas vegetais disponíveis localmente. No entanto, a ocorrência de fatores antinutricionais na farinha de plantas suprime seu potencial de ser totalmente substituída. Portanto, neste estudo objetivamos estudar os efeitos sinérgicos de aditivos dietéticos como ácido cítrico e suplementação com enzima fitase sobre o desempenho de crescimento e digestibilidade de nutrientes de alevinos de Cirrhinus mrigala. A farinha de canola (CM) foi usada como ingrediente de teste para substituir a farinha de peixe (FM) como: 0%, 25%, 50% e 75%. Essas quatro dietas foram suplementadas por níveis variados de fitase (0 e 750 FTU kg^{-1}) e ácido cítrico (0% e 2,5%) para formular um total de 16 dietas de teste como T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, T12, T13, T14, T15 e T16. Cada tratamento continha três repetições; aplicado a grupos de peixes com 15 alevinos cada; seguindo o arranjo fatorial 3 × 3. 1% de óxido crômico foi adicionado como um marcador inerte. % de ganho de peso máximo (288%) e o valor mais baixo de FCR (1,07) foram registrados quando os peixes foram alimentados com dieta T12 em comparação com peixes alimentados com dieta controle (T1). Da mesma forma, valores ótimos de digestibilidade de nutrientes, como proteína bruta (77%), gordura bruta (84%) e energia bruta (70%) foram anotados no mesmo nível. Concluiu-se que 50% da farinha de canola pode substituir de forma ideal a farinha de peixe quando suplementada com fitase e ácido cítrico nos níveis de 750 FTU kg^{-1} e 2,5%, respectivamente.

Palavras-chave: *C. mrigala*, crescimento, digestibilidade de nutrientes, farinha de peixe, farelo de canola.

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1. Introduction

Fishmeal is a vital ingredient being used in fish feed formulation (Salin et al., 2018). It is a main source of nutrients such as essential vitamins, amino acids, fatty acids and trace minerals. The intensive use of fishmeal has affected its protein quality as well as the cost. The need to replace fishmeal in aqua-feed is mandatory because the sustainability of boosting aquaculture industry requires the decrease in wild fish capture (Alhazzaa et al., 2019). Suitable plant feed ingredients such as grains and oilseed by-products meal are the most promising sources of protein and energy for feed formulation in the future (Manuel et al., 2019). Studies proved that fishmeal can be replaced up to 30% by an equal mixture of cottonseed and soybean meal; with iron and phytase in the presence of lysine and methionine in fish feed (Lim and Lee, 2009).

Canola meal (CM) is a nutritionally important feed ingredient derived from *Brassica napus*, containing 33-45% protein contents (Glencross, 2016). It is less expensive than FM and ubiquitous in nature. It has met an elevated global production since last years. Being a highly suitable plant-based source of protein, it is incorporated as a major protein source in feed formulation (Mushtaq et al., 2006). The oilseed by-products meals are increasingly important in preparation of economical and environment friendly fish feed (Cheng and Hardy, 2002) but one major problem associated with the use of plant protein is the presence of anti-nutritional factors such as phytate, fibers and glucosinolates (von Danwitz and Schulz, 2020).

It is estimated that up to 80% of the total phosphorus contents in plants are present in the form of phytate and is practically not available for monogastric or agastric animals (NRC, 1993); due to lack of intestinal phytase (PHY) for efficient phytate hydrolysis during digestion (Jackson et al., 1996). It also makes a bond with vitamins and proteins and reduces their utilization, absorption and interferes with starch and lipid digestibility (Papatryphon et al., 1999). Studies have shown the efficacy of PHY as growth enhancer in species such as carps and Nile tilapia (Phromkunthong et al., 2010; Olusola and Nwanna, 2014).

It has been reported that citric acid (CA) is being successfully used to improve growth performance, nutrients utilization and resistance against diseases due to its phytate hydrolyzing capability (Dai et al., 2018). Being a chelating agent, it also works as a binder with various types of cations in the intestine and makes them easy to be absorbed by lowering the gut pH and improve bone mineralization (Shafique et al., 2018). A number of feeding trials with promising responses have been conducted to evaluate the effect of CA acidified diets on growth, feed intake and feed performance of fish (Pandey and Satoh, 2014; Bano and Afzal, 2018).

C. mrigala commonly known as "mori" is one of the major Indian carp species; cultured in Pakistan (Hussain et al., 2011). It is bottom feeder and feed on decaying organic matter and vegetable debris. For the success of carp farming, the use of cost-effective feed has become necessary to control the farm's economy (Abid and Ahmed, 2009). So, it is necessary to introduce nutritionally balanced diet as a limiting factor for carp's optimal growth (Khan et al., 2004). Therefore, the present study was conducted to investigate the effect of CA and PHY supplementation on growth performance and nutrient digestibility of *C. mrigala* fingerlings fed CM based diets as well as to help the aqua-culturists in producing environment friendly and cost-effective fish.

2. Materials and Methods

The current experiment was conducted in Fish Nutrition Laboratory, Department of Zoology, Government College University Faisalabad, Pakistan (Latitude 31.4166° North and the Longitude 73.0707° East).

2.1. Experimental design

A 90 days, 3×3 factorial experiment was conducted by using canola meal as a test ingredient. Four test diets were formulated by replacing fishmeal with canola meal at levels of 0%, 25%, 50% and 75%. These four diets were further supplemented by varying levels of phytase (0 and 750 FTU kg^{-1}) and CA (0% and 2.5%); to formulate total sixteen test diets, designated 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}. Each treatment contained three replicates having 15 fingerlings (initial weight; 6.570±0.053g) in each triplicate tank, hence a total of 720 fingerlings were stocked.

2.2. Feed formulation

Feed ingredients including CM were procured from the local market and their chemical composition was analyzed before the formulation of the test diets by standard methods (AOAC, 2005). All the feed ingredients were grinded finely to pass through (0.5 mm) mesh size (Table 1). An electric mixer was used to mix all feed ingredients for about 10-20 minutes and fish oil was added gradually while mixing. 15% moisture was added by adding distilled water and pellets were made by using pelleting machine (Lovell, 1989). Preceding the pellet formation, phytase was supplemented by spraying method (Jackson et al., 1996). The pellets were then dried at room temperature and refrigerated at 4 °C, until the start of feeding trial.

2.3. Experimental facility and fish feeding

C. mrigala fingerlings were obtained from Government Fish Seed Hatchery, Faisalabad. The fingerlings were acclimatized and stocked in V-shaped tanks with proper aeration provided in the laboratory conditions. During the period of acclamation, the fish were fed on basal diet once daily (Allan and Rowland, 1992). Before beginning of experiment, *C. mrigala* fingerlings were given the dip prophylactically in NaCl solution (5g/L) to remove the ectoparasites and to avoid from any fungal infection (Rowland and Ingram, 1991). During this experiment, triplicate tanks were allotted for each treatment. Fingerlings were fed by hand till apparent satiation at 07:00 a.m.; at a ration equal to 5% of its live wet weight. Uneaten feed was collected after an hour of feeding session and oven dried at 60°C for feed consumption analysis. After that, experimental tanks were washed and refilled with tap water. All tanks were aerated round the clock and water quality parameters were monitored and maintained by using physical equipment; including thermometer for temperature, DO meter (Jenway 970) for dissolved oxygen and pH meter (Jenway 3510) for pH measurements.

2.4. Chemical analysis of feed and feces

Feed ingredients, samples of prepared test diets and feces were homogenized with the help of motor and pestle, then subsequently analyzed by the given standard methods. Moisture was determined at 105 °C for 12h by oven drying method, crude protein (N × 6.25) by micro-Kjeldahl apparatus, crude fat (Soxtec HT2 1045 system) by ether extraction method, crude fiber by loss on ignition and gross energy by oxygen bomb calorimeter.

2.5. Growth study

Variation in the growth parameters of *C. mrigala* fingerlings was estimated at the beginning and end of the experiment by following standard methods (Hussain et al., 2015) (Equations 1-3).

$$Weight\ gain\ \% = \frac{(Final\ weight\ -\ Initial\ weight)}{Initial\ weight} \times\ 100 \quad (1)$$

$$FCR = \frac{Total\ dry\ feed\ intake\ (g)}{Wet\ weight\ gain\ (g)} \quad (2)$$

$$SGR = \frac{Final\ Weight\ (g)\ -\ Initial\ Weight(g)}{No\ of\ trial\ days} \times 100 \quad (3)$$

2.6. Digestibility study

Preceding the oxidation with molybdate reagent, chromic oxide contents in diets and feces were estimated using UV-VIS 2001 spectrophotometer at 370nm absorbance (Divakaran et al., 2002). Apparent nutrient digestibility coefficient (ADC%) for experimental diets was calculated by the standard formula (NRC, 1993) (Equation 4).

$$ADC(\%) = 100 - 100 \times \frac{\%\ marker\ in\ diet\ \times\ \%\ nutrient\ in\ feces}{\%\ marker\ in\ feces\ \times\ \%\ nutrient\ in\ diet} \quad (4)$$

2.7. Statistical analysis

The individual and interactive effects of CA and PHY were calculated by applying three-way analysis of variance on obtained data. The results were considered significant at $p<0.05$ (Snedecor and Cochran, 1991). The CoStat computer package (Version 6.303, PMB 320, Monterey, CA, 93940 USA) was used for statistical analysis.

3. Results

3.1. Growth performance

Table 2 shows the growth parameters of *C. mrigala* fingerlings fed on PHY and CA supplemented CM based diet. It was recorded that both the supplementations individually brought significant changes in improving growth performance of *C. mrigala* fingerlings. Dietary acidification through the inclusion of CA lowered the pH of the fish gut in dose dependent manner and improved the growth parameters ($p<0.05$). Phytate hydrolysis was maximum when both the supplements interacted synergistically at T_{12} diet; having 50% FM substituted with CM supplemented with 2.5% CA and 750 FTU kg^{-1} level of phytase supplementation. Significantly ($p<0.05$) improved weight gain (WG) (18g), weight gain% (WG%) (288g), survival rate (100%) and lowest FCR (1.07) was noted at this level which was different from the fish fed on control diet and all other supplemented levels as shown in Figures 1 and 2. Second best results were noted down in T_{11}; in which 750 FTU kg^{-1} PHY rendered the WG% and FCR to 253% and 1.21, respectively. The lowest values of growth indices such as WG (9g), WG% (156%), FCR (2.02) and survival (96%) were observed in the fish fed on T_1 test diet, having more inclusion of FM than the CM with 0% CA and 0 FTU kg^{-1} level of PHY supplementation. The growth parameters started to increase positively with the increase in CM along with CA and PHY interactive level and then changed inversely with the further increase in CM level.

Table 1. Ingredients composition (%) of test diets.

Test diets	Fishmeal Protein Replacement Levels (%)	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	14.5/15	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

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

Test diets	Fishmeal Protein Replacement Levels (%)	PHY (FTU kg^{-1})	CA (%)	Initial weight	Final weight	Weight gain	SGR	Survival%
T_1	0%	0	0	6.35±0.040	16.30±0.654^{j}	9.95±0.618^{i}	1.04±0.038^{b}	96
T_2			2.5	6.500±0.026	17.813±0.428^{i}	11.313±0.450^{h}	1.11±0.030^{b}	96
T_3		750	0	6.570±0.040	18.743±0.585^{h}	12.173±0.613^{g}	1.16±0.039^{b}	98
T_4			2.5	6.510±0.040	21.023±0.912f	14.513±0.899^{e}	1.30±0.045^{b}	98
T_5	25%	0	0	6.543±0.042	17.853±0.465^{i}	11.310±0.426^{h}	1.11±0.022^{b}	96
T_6			2.5	6.557±0.122	20.230±0.969^{g}	13.673±1.084^{f}	1.25±0.072^{b}	98
T_7		750	0	6.570±0.053	21.150±0.350^{f}	14.580±0.367^{e}	1.29±0.022^{b}	98
T_8			2.5	6.597±0.101	22.223±0.665^{d}	15.627±0.565^{d}	1.34±0.016^{b}	100
T_9	50%	0	0	6.523±0.091	18.670±0.901^{h}	12.147±0.986^{g}	1.16±0.068^{b}	96
T_{10}			2.5	6.570±0.050	19.930±0.527^{g}	13.360±0.490^{f}	1.23±0.023^{b}	98
T_{11}		750	0	6.563±0.092	23.397±1.181^{b}	16.787±1.110^{b}	1.40±0.045^{b}	100
T_{12}			2.5	0.040±0.040	25.403±0.344^{a}	18.870±0.310^{a}	1.50±0.009^{a}	100
T_{13}	75%	0	0	6.580±0.082	19.737±0.558^{g}	13.157±0.600^{f}	1.22±0.039^{b}	96
T_{14}			2.5	6.660±0.050	21.590±0.598^{e}	14.930±0.598^{e}	1.30±0.031^{b}	100
T_{15}		750	0	6.563±0.076	21.690±0.486^{e}	15.127±0.478^{e}	1.32±0.025^{b}	100
T_{16}			2.5	6.577±0.049	22.810±0.688^{c}	16.233±0.653^{c}	1.38±0.027^{b}	100

Means within columns having different superscripts are significantly different at $p<0.05$. Data are means of three replicates. SGR; Standard Growth Rate.

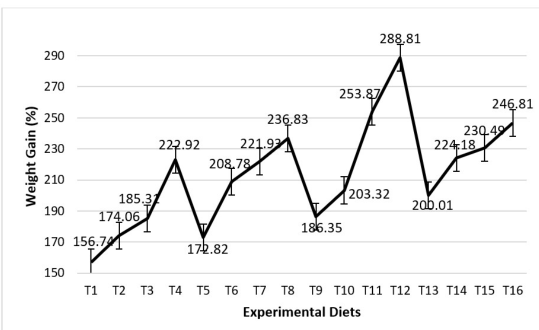

Figure 1. Weight gain (%) of *C. mrigala* fingerlings fed on CM based diets supplemented with PHY and CA.

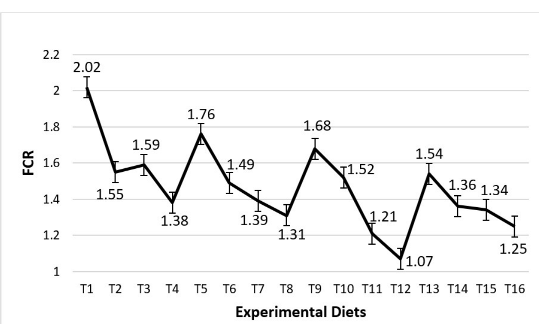

Figure 2. FCR of *C. mrigala* fingerlings fed on CM based diets supplemented with PHY and CA.

3.2. Apparent nutrient digestibility

the nutrients percentage in feed and feces of *C. mrigala* fingerlings and its digestibility values are given in Tables 3, 4 and 5, respectively. Notably highest ADC (%) (CP: 77%, CF: 84% and GE: 70%) were observed in the fish group fed test diet T_{12} while the lowest values of nutrients digestibility (CP: 51%, CF: 65% and GE: 53%) were observed in the fish fed with control test diet (T_1) showing that simultaneous supplementation of phytase and citric acid (CA×PHY) in canola meal based diets enhanced the nutrient digestibility by degrading the phytate complexes. By breakdown of phytate, the essential nutrients were released and became available for the fish to fulfill its nutritional demands. Individual role of phytase at 750 FTUkg^{-1} was also significant (T_{11}) and brought CP, CF, and GE to 76%, 83% and 68%, respectively. Results of present work also suggested that high dose of phytase level i.e., 750 FTU kg^{-1} when combined with optimum level of CA (2.5%), resulted in improved nutrient digestibility (Table 5). Due to increased nutrient digestibility of diet, growth performance was also enhanced. So, highly significant ($p<0.05$) differences in digestibility of nutrients of the (T_1) and that of the treated levels can be observed. Hence, it can be deduced from the above mentioned results that nutrient digestibility parameters were improved when both the supplements (CA×PHY) interacted synergistically with increasing CM levels but up to 50%, afterwards it showed inverse relationship.

4. Discussion

When CA is used in combination with PHY in plant meal based fish diet, the release of H^+ ions show positive results by reducing the pH of the stomach, breaking down the PHY complexes and intensifying the PHY activity (Shah et al., 2015, 2021). In the current study, the maximum values of growth performance for *C. mrigala* fingerlings were noted at 2.5% CA and 750 (FTU kg^{-1}) PHY supplementation in diets having 50% FM substitution level. Likewise, Mahmoud et al. (2019) and Nehad et al. (2020) found significantly improved ($p<0.05$) growth performance (WG%: 67%, FCR: 1.50) and feed utilization parameters while studying interactive effects of CA and PHY at 30 g/kg and 1000 FTUkg^{-1}, respectively in *Oreochromis niloticus*. This is in agreement with Baruah et al. (2007), who found maximum growth performance at 3% CA and 500 (FTU kg^{-1})

Table 3. Analyzed compositions (%) of apparent crude protein, crude fat and gross energy in the diets of *C. mrigala* 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	3.4±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±0.02
T_{11}		750	0	30.62±0.03	8.04±0.010	3.41±0.02
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±0.04

Data are means of three replicates.

Table 4. Analyzed compositions (%) of apparent crude protein, crude fat and gross energy in feces of *C. mrigala* 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	15.72±0.448^{a}	2.24±0.025^{a}	1.69±0.015^{a}
T_2			2.5	14.01±0.065^{c}	2.01±0.036^{c}	1.6±0.01^{c}
T_3		750	0	13.34±0.0361^{d}	1.94±0.032^{d}	1.58±0.01^{d}
T_4			2.5	10.55±0.025^{j}	1.40±0.015^{j}	1.39±0.015^{j}
T_5	25%	0	0	14.68±0.065^{b}	2.1±0.02^{b}	1.63±0.011^{b}
T_6			2.5	11.36±0.061^{h}	1.52±0.025^{h}	1.46±0.015^{h}
T_7		750	0	10.95±0.047^{i}	1.47±0.015^{i}	1.42±0.015^{i}
T_8			2.5	8.92±0.035^{m}	1.76±0.036^{e}	1.30±0.011^{m}
T_9	50%	0	0	12.92±0.055^{e}	1.75±0.03^{e}	1.56±0.026^{e}
T_{10}			2.5	11.75±0.03^{g}	1.57±0.026^{g}	1.49±0.02^{g}
T_{11}		750	0	7.96±0.0378^{o}	1.08±0.015^{m}	1.19±0.015^{o}
T_{12}			2.5	7.7±0.075^{p}	1.02±0.01^{n}	1.12±0.025^{p}
T_{13}	75%	0	0	12.14±0.050^{f}	1.64±0.015^{f}	1.53±0.015^{f}
T_{14}			2.5	10.03±0.06k	1.76±0.036^{e}	1.36±0.02^{k}
T_{15}		750	0	9.43±0.035^{l}	1.28±0.032^{k}	1.33±0.015^{l}
T_{16}			2.5	8.21±0.035^{n}	1.14±0.02^{l}	1.26±0.015^{n}

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

PHY supplementation level. Similarly, Zhu et al. (2014) and Thiam et al. (2015) reported that the interaction of CA with PHY worked best to improve growth and protein content of yellow catfish and Nile tilapia fingerlings, respectively. A recent study conducted by Fadhal and Mustafa (2020) confirmed the role of phytase as a growth promoting dietary additive when used at 4000IU/kg in the diet of common carp. Similar results of optimized growth were reported by Hussain et al. (2015) but the levels were different from our study. They recorded that supplementation of 5% CA and 500 (FTU kg^{-1}) PHY resulted in the maximum growth (WG%: 401% and FCR: 1.21) of *C. mrigala* fingerlings fed canola meal-based diet. In recent researches, it was found that incorporation of CA and PHY in fish feed must be around 2.5-5% and 250-1500 FTUkg^{-1} (Cao et al., 2007; Khajepour et al., 2012).

Table 5. Apparent Digestibility Coefficient (ADC%) of crude protein, crude fat and gross energy of *C. mrigala* 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	51.68±0.522^{o}	65.25±0.488^{o}	53.51±0.948^{p}
T_2			2.5	54.85±2.383^{n}	49.30±10.047^{p}	55.97±1.018^{n}
T_3		750	0	59.28±0.654^{m}	70.16±0.842^{m}	56.63±0.665^{m}
T_4			2.5	67.47±0.566^{g}	78.22±0.209^{g}	61.72±0.686^{g}
T_5	25%	0	0	54.73±0.398^{n}	67.29±0.203^{n}	55.01±0.307^{o}
T_6			2.5	65.47±1.084^{i}	76.66±0.412^{i}	60.45±1.709^{i}
T_7		750	0	66.45±0.862^{h}	77.37±0.346^{h}	60.91±1.694^{h}
T_8			2.5	73.39±0.505^{d}	81.07±0.259^{d}	65.33±0.287^{d}
T_9	50%	0	0	61.22±1.328^{l}	73.39±0.452^{l}	57.99±1.755^{l}
T_{10}			2.5	64.47±0.369^{j}	75.62±0.136^{j}	59.61±0.839^{j}
T_{11}		750	0	76.61±0.314^{b}	83.97±0.009^{b}	68.74±0.414^{b}
T_{12}			2.5	77.38±0.340^{a}	84.81±0.063^{a}	70.47±0.400^{a}
T_{13}	75%	0	0	63.05±0.884^{k}	74.71±0.602^{k}	58.16±0.829^{k}
T_{14}			2.5	69.73±0.315^{f}	79.50±0.193^{f}	63.29±0.130^{f}
T_{15}		750	0	71.58±0.249^{e}	80.41±0.408^{e}	64.12±0.505^{e}
T_{16}			2.5	75.63±0.358^{c}	82.79±0.167^{c}	66.68±0.116^{c}

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

Data in the present study proved that a significant difference exist 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. The findings support the hypothesis that addition of organic acid in the diet gives the favorable environment to PHY for optimum performance. Shah et al. (2021) recorded the improved nutrient utilization and hence improved growth of *L. rohita* fingerlings by using combined effect of PHY and CA at 1000 FTUkg^{-1} and 30 g/kg in sunflower meal based diets. Both of these supplements show synergistic effect of phytate hydrolysis when used in combination. This difference in concentration can be due to varied experimental conditions and test species. 20 g/kg optimum supplementation level. To conclude, the supplementation of this organic acid in diets does not negatively affect fish nutritional status, health or welfare, and increases nutrient and mineral availability. Nascimento et al. (2021) also found increased ADC (%) for crude energy and dry matter when fed *Colossoma macropomum* on plant based diet supplemented with 18.5g/kg citric acid. Similar to our results, positive results of growth performance and feed utilization were found by different researchers on a number of species including *C. mrigala* (Hussain et al., 2017), tilapia (Maas et al., 2018) and *L. rohita* (Bano and Afzal, 2018).

Results of the present study are also supported by Hussain et al. (2011) who stated that 750 (FTU kg^{-1}) PHY supplementation level with plant-meal based diet is optimal for highest growth performance of *L. rohita* fingerlings. In contrast to our study, Khajepour and Hosseini (2012) reported that the addition of citric acid in the fish diet reduced the digestibility of lipid because he claimed a decrease in fat digestibility with PHY addition. Similarly, contrasting results of non-significant change in growth rate, feed intake or feed efficiency was recorded by Dai et al. (2018) in turbot. In the same way, Yigit et al. (2018) also reported the non-significant effect of phytase supplementation in soybean meal based diets even at 2 g/kg level, resultantly growth parameters and feed utilization parameters remain unaffected. Its possible reason can be different dietary factors such as sources and concentration of phytase in the diet, type of feed ingredients, methods of fish drying, age and size of fish and fish species.

5. Conclusion

Under the experimental conditions tested herein, it can be said that addition of CA and PHY in CM based diets enhanced digestibility of nutrients for *C. mrigala* fingerlings, which in turn led to the improved growth performance. It was also deduced that test diet T_{12} having 50% fishmeal substituted with canola meal supplemented with 2.5% CA and 750 FTU kg^{-1} PHY level proved to be the optimum diet for the improvement of the all above said factors. Acidification of PHY with CA proved to be efficient in formulating nutritionally bioavailable, cost effective and eco-friendly feed for *C. mrigala*. Besides, there is need of detailed study on different size and age group of fish.

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