Efficacy of phytase enzyme and citric acid on growth performance, nutrients and mineral digestibility of Cirrhinus mrigala fingerlings fed guar meal-based diet

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Hussain S.M.¹*; Ahmad N.¹; Shahzad M.M.²; Javid A.³; Aslam N.⁴; Hussain M.⁵; Arsalan M.Z.H.¹; Riaz D.¹

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Abstract

The study was carried out to estimate the effects of phytase, and citric acid (CA) supplemented guar meal based diet on growth performance, nutrients and mineral digestibility in Cirrhinus mrigala fingerlings. The experiment consisted of nine test diets. Diets were formulated by spraying graded levels of phytase (0, 500 and 1000 FTU kg⁻¹) and 0% (0g), 2.5% (75g) and 5% (150g) citric acid supplementation to a guar meal-based diet. Chromic oxide was added as an indigestible marker. Fingerlings were fed at the rate of 5% of live wet weight. The maximum growth performance, minerals, and nutrients digestibility value were observed in fingerlings fed diet supplemented with 1000 FTU kg⁻¹ level of phytase and 2.5% CA. These values were significantly different from fish fed the control and other test diets. It was concluded that the phytase and CA supplementation to a guar meal based diet at 2.5% CA and 1000 FTU kg-1 level is optimum to release sufficient amount of chelated minerals and nutrients for C. mrigala fingerlings. Our findings also suggested that phytase and CA supplementation can help in the development of sustainable aquaculture by reducing the feed costs and nutrient discharge through feces into the aquatic ecosystem.

Keywords: Cirrhinus mrigala, Phytase, Citric acid, Guar meal, Growth, Nutrient digestibility.

¹⁻Fish Nutrition Lab, Department of Zoology, Government College University, Faisalabad, Pakistan

²⁻Department of Zoology, Division of Science and Technology, University of Education Township, Lahore

³⁻Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore, Pakistan

⁴⁻Department of Biochemistry, Government College University, Faisalabad, Pakistan

⁵⁻Department of Zoology, University of Gujrat, Gujrat, Pakistan

^{*}Corresponding author's Email: drmakhdoom90@gmail.com

Introduction

Cirrhinus mrigala commonly known as "mori," one of the major carp species cultured in Pakistan, is a bottom feeder and feeds on vegetable debris and decaying organic The matter. aquaculture industry is expanding very rapidly to fulfill the need for highprotein quality fish for human nutritional requirements (Tacon and Metian, 2008; Naylor et al., 2009; Yıldırım et al., 2014). Both the feed costs and feed efficiency are primary factors which control the fish farm economy (Khajepour et al., 2012a).

In the past fish feed was reliant on the use of fishmeal as a source of essential nutrients and growth factors because fish meal is enriched with essential nutrients such as indispensable fatty acids, essential amino acids, vitamins and many growth factors (NRC, 1993; Zhou et al., 2004). Due to increasing demand, rising prices and uneven supply of fish meal made it necessary to search for alternative protein sources for the fish feed industry (Pham et al., 2008; Lech and Reigh, 2012). Nowadays one of the most important challenges to the aquaculture industry is the formulation of cost-effective fish feed from better quality protein sources (Baruah et al., 2004). The main objective for most fish farmers is to produce high-quality fish feed at low costs because feeds primarily account for 50 to 60% of the total cost in fish culture (Essa et al., 2004).

Some researchers have found positive effects of plant meal on fish growth (Hussain *et al.*, 2011). So the

of plant meal has use been recommended as an alternative protein source for fishmeal, as these cheaper, easily available and environment-friendly because pollution causing contents of plant-based feeds are lower than that of the fishmeal (Dalsgaard et al., 2009). Of these plant meal meals, guar is relatively inexpensive with high protein contents (33 to 45%). Guar meal is a good binding agent in feed formulation. It is also important that guar meal is free from salmonella, E. coli, and aflatoxin (Nagpal et al., 1971). But unfortunately, it has low minerals availability due to the presence of the anti-nutritional factor i.e. phytate or phytic acid (Liu et al., 2013). It has highly adverse effects on the fish digestive tract and results in poor growth performance (Baruah et al., 2004). Phytic acid is the main form of storing phosphorus in plant seeds (Jorquera et al., 2008) and usually, it minimum availability causes minerals and decreases the apparent digestibility of nutrients and minerals (Gatlin et al., 2007). Phytate has been historically considered an antinutritional factor because it is known as a strong chelator of divalent minerals such as Ca, Mg, Zn and Fe (Noureddini and Dang, 2008).

Bioavailability of dietary nutrients and minerals is also greatly influenced by acidification through organic acids in several ways. Firstly, they modify the mineral transport mechanism by altering the gastric acidity. Secondly, they decrease the chelating and complex forming ability of elements (Ravindran and Kornegay, 1993). Thus they ensure the increased absorption of phosphorus (P) and other elements. Thirdly, these organic acids the proliferation of the stimulate epithelial cells in gastrointestinal mucosa (Sakata et al., 1995) thereby increasing the absorption area for nutrients and minerals (Baruah et al., 2007a). Among these organic acids, citric acid (CA) has been extensively used for diet acidification due to its unique flavor and high buffering capacity (Hossain et al., 2007). Chemically, it is known as 2-Hydroxy-1, 2, 3- Propane tri-carboxylic acid with the chemical formula (COOH)₃(CH₂)₂C(OH). It has good water solubility, and its gross energy is 2460 kcal kg⁻¹. The diets supplemented with CA, increased the retention of Ca, Mg, Na, K, Zn, and Mn in Yellowtail (Sarker et al., 2012 a,b). It also enhanced nitrogen retention in red sea bream with its different supply levels in the diet (Sarker et al., 2006). Baruah et al. (2007a) found that the addition of improved the absorption CA minerals (Na, P, K, Mn, Mg, Fe, Cu, Ca and N) and their concentration in plasma and the whole body of Rohu. The increment in carcass mineral deposition suggests that the organic acids and other supplements enhanced the mineral utilization of dietary fish meal and plant protein meal (Hossain et al., 2007). Citric acid increases the bioavailability of minerals in several ways. It solubilizes the bones present in the meal and releases the chelated nutrients and minerals (Sarker et al., 2006). Being a strong chelator of Ca

and P, it removes these minerals from the phytate; making it less stable and susceptible to endogenous phytases (Khajepour and Hosseini, 2010). Moreover, CA increases the bioavailability of minerals by competing with the dietary mineral inhibitors. The addition of microbial and CA enhances phytase availability of phosphorus from plant sources, improves bone mineralization, growth and feed efficiency. Combining a low dose of CA to the phytase diets significantly supplemented increased the positive effects of the enzyme (Phromkunthong et al., 2010). The objective of the present study was to investigate the effects of phytase, and citric acid supplementation on nutrients and mineral digestibility in C. mrigala fingerlings fed a guar meal based diet with the aims of growth estimation.

Materials and methods

The study was conducted in the Fish Nutrition Laboratory, Department of Zoology Government College University Faisalabad.

Fish and experimental conditions Cirrhinus mrigala fingerlings were purchased from the Fish Seed Hatchery, Satiana Road. Faisalabad. The fingerlings were stocked in V-shaped fish tanks (GCUF system) that were specially designed for the collection of fecal material. **Fingerlings** were acclimatized in the laboratory to experimental conditions for 15 days. During the acclimatization period fish were fed twice daily to apparent satiation on the basal diet, used in subsequent digestibility studies (Allan and Rowland, 1992). Water quality parameters particularly water рН and temperature; DO were monitored on a daily basis. Aeration was provided 24 hours a day to fish throughout the study period. NaCl (5 g L⁻¹) was used on *C. mrigala* fingerlings for the treatment of ectoparasites as well as to prevent fungal infection, before starting the experiment.

Experimental design

Guar meal was used as the test ingredient to formulate the diets. experimental This diet was divided into three groups, each of 3 kg weight. The Guar meal based diet was treated with different concentrations of CA and phytase to formulate nine test diets such as T1: 0% CA, 0 FTU kg⁻¹, T2: 0% CA, 500 FTU kg⁻¹, T3: 0% CA, 1000 FTU kg⁻¹, T4: 2.5% CA, 0 FTU kg⁻¹, T5: 2.5% CA, 500 FTU kg⁻¹ T6: 2.5% CA, 1000 FTU kg⁻¹, T7: 5% CA, 0 FTU kg⁻¹, T8: 5% CA, 500 FTU kg⁻¹ and T9: 5% CA, 1000 FTU kg⁻¹. These test diets supplemented with CA and phytase were fed to nine groups of fish stocked in the experimental tanks. Each of the treatments and control diets had three replicates with 15 fingerlings in each replicate. The total duration of the experiment was 90 days. Guar meal based CA and phytase supplemented diets were compared with each other to determine growth, nutrient and mineral digestibility parameters using Completely Randomized Design (CRD).

Feed ingredients and experimental diets The feed ingredients (Table 1) were purchased from the local market and analyzed were for chemical composition following AOAC (1995) before the formulation of the experimental diets (Table 2). The feed ingredients were finely ground to pass through a 0.5 mm sieve. All ingredients were mixed in an electric mixer for 10 min, and fish oil was gradually added while mixing the ingredients. percent water was also added to form suitable dough and it was extruded using a SYSLG30-IV experimental extruder to produce pellets (3 mm). The above procedure was followed to formulate the 9 guar meal based test diets. 0g (0%), 75g (2.5%) and 150g (5%)CA was added to them respectively. The required concentrations (0, 500 and 1000 FTU kg⁻¹) of phytase (Phyzyme® XP 10000 FTU g-1; Danisco Animal Nutrition, Fin-65101 Finland) Vaasa. were prepared in 25 mL distilled water and sprayed on 1 kg of test diets (Robinson et al., 2002). The control diet (0FTU kg⁻¹) was sprayed with an equal volume of distilled water to maintain similar moisture contents. All the prepared diets were dried and stored at 4°C before use.

Table 1: Ingredients composition (%) of guar meal based diets.

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Ingredients	Test diet-I	Test diet-II	Test diet- III	Test diet- IV	Test diet-V	Test diet- VI	Test diet-VII	Test diet- VIII	Test diet-IX
Citric acid	0	2.5	5	0	2.5	5	0	2.5	5
Fish meal	12	12	12	12	12	12	12	12	12
Guar meal	56	56	56	56	56	56	56	56	56
Rice polish	12	12	12	12	12	12	12	12	12
Wheat flour	10	7.5	5	10	7.5	5	10	7.5	5
Fish oil	6	6	6	6	6	6	6	6	6
Vitamin premix	1	1	1	1	1	1	1	1	1
Minerals	1	1	1	1	1	1	1	1	1
Ascorbic acid	1	1	1	1	1	1	1	1	1
Chromic oxide	1	1	1	1	1	1	1	1	1
Phytase* FTU kg ⁻¹	0	0	0	500	500	500	1000	1000	1000

^{*}Phytase enzyme was used at the expense of wheat flour

Table 2: Chemical composition (%) of feed ingredients (Dry matter basis).

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Ingredi ents	Dry matter (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	Ash (%)	Gross Energy (kcal g ⁻¹)	Carbohydrates
Fish meal	91.63	48.15	7.06	1.09	24.73	2.13	16.84
Wheat flour	92.45	10.10	2.35	2.65	2.08	2.96	79.86
Guar meal	89.00	36.50	3.50	11.00	11.10	2.00	35.90
Rice polish	94.09	12.25	13.44	12.60	10.15	3.33	48.23

Feeding protocol and sample collection Cirrhinus mrigala fingerlings were fed two times day (morning At the start afternoon). the experiment the fish fingerlings were fed at the rate of 5% of live wet weight on their prescribed diet and later on adjusted to a daily basis intake of feed by fish. For each test diet, three replicates were used, and in each replicate, 15 fingerlings (average weight: 8.02 g fish⁻¹) were stocked. From each tank, the uneaten diet was drained out after the feeding period of two hours. Before refilling the water, the tanks were washed completely to remove the particles of uneaten diets.

Feces were collected from the fecal collection tube of each tank after two hours. Fecal material of each replicate treatment was dried in an oven and stored for further chemical analysis.

Growth study

Fish in each tank were bulk weighed every 15th day during the experiment to assess growth performance of *C. mrigala* fingerlings. Weight gain (%) and feed conversion ratio (FCR) of fingerlings were evaluated based on standard formulae.

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

Initial weight
FCR= Total dry feed intake (g)
Wet weight gain (g)

Chemical analysis of feed and feces

The samples of feed ingredients, experimental diets and feces were homogenized using a motor and pestle and then analyzed by standard methods (AOAC. 1995). Moisture was determined by oven drying at 105°C for 12h; crude protein (N \times 6.25) by micro-Kjeldahl apparatus; crude petroleum ether extraction method through Soxtec HT2 1045 system; crude fiber as loss on ignition of dried lipid-free residues after digestion with 1.25% H₂SO₄ and 1.25% NaOH; Ash, by ignition at 650°C for 12 h in electric furnace (Eyela-TMF 3100) to constant weight. Gross energy was determined with the help of oxygen calorimeter.

Chromic oxide estimation

Chromic oxide was used as an inert marker in diets assuming that the amount of the marker in the feed and feces remains constant throughout the experimental period and the entire ingested marker appears in the feces. Chromic oxide contents in diets and feces were estimated after oxidation with molybdate reagent (Divakaran et 2002) using **UV-VIS** 2001 spectrophotometer 370nm at absorbance.

Minerals analysis of feed and feces

For mineral estimation, the diets and feces samples were digested in boiling nitric acid and perchloric acid mixture

(2:1) by following standard methods (AOAC, 1995). After appropriate dilution, mineral contents (calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn) and manganese, (Mn) were estimated using atomic absorption (Hitachi Polarized Atomic Absorption Spectrometer, Z-8200). Calibrated mineral standards for estimation were prepared from commercially available standards (AppliChem® Gmbh Ottoweg4, DE-64291 Darmstadt, Germany). sodium estimation of (Na) and potassium (K) was done through flame PFP-7, photometer (Jenway UK). Phosphorus (P) was analyzed calorimetrically (UV/VIS spectrophotometer) using ammonium molybdate as a reagent at 720 nm absorbance using standard methods (AOAC, 1995).

Calculation of digestibility

Apparent nutrient digestibility coefficients (ADC%) of experimental diets were calculated by the formula reported in NRC (1993).

ADC (%)= $100-100 \times Percent marker in diet \times Percent nutrient in feces$

Percent marker in feces×Percent nutrient in diet

Statistical analysis

Finally, data of nutrient digestibility of experimental diets was subjected to two-way analysis of variance, ANOVA (Steel *et al.*, 1997). The differences among means were compared by Tukey's Honesty Significant Difference Test and considered significant at p < 0.05 (Snedecor and Cochran, 1990).

The CoStat-computer package (Version 6.303, PMB 320, Monterey, CA, 93940 USA) was used for statistical analysis.

Results

From the present results, it was found that phytase and CA supplementation increased the minerals and nutrient availability to fish resulted in higher growth performance. The highest digestibility of minerals and nutrients, as well as growth performance, was observed in fish fed test diets supplemented with 2.5% CA and 1000 FTU kg^{-1} diet (T_6).

Cirrhinus mrigala fingerlings fed on phytase and CA supplemented guar meal based diets showed improved

weight gain, weight gain (%) and FCR as compared to the control diet. The maximum weight gain (29 g), weight gain % (418 %) and the best FCR value (1.03) of C. mrigala fingerlings was noted on the guar meal based diet having 2.5% CA and 1000 FTU kg⁻¹ level supplementation (T_6) . The second highest weight gain (28 g), weight gain % (403%) and the best FCR value (1.24) were observed when fingerlings were fed on the guar meal based diet supplemented with 5% CA and 1000 FTU kg⁻¹ level. These values were significantly (p<0.05) different from the control diet (weight gain (23 g), weight gain% (304 %) and FCR value (2.09) as shown in Table 3.

Table 3: Growth performance of *Cirrhinus mrigala* fingerlings fed on CA and phytase supplemented guar meal based diet.

Treatments	CA (%)	Phytase level (FTU kg ⁻¹)	Initial weight	Final weight	Weight gain	Weight gain %	Weight gain (fish ⁻¹ day ⁻¹) g	Feed intake (fish ⁻¹ day ⁻¹) g	FCR
T_1		0	5.62	22.69	17.07	304.03	0.24395	0.51040	2.09
T ₂	0	500	5.63	24.07	18.44	327.83	0.26352	0.47267	1.79
T ₃		1000	5.62	25.15	19.53	347.34	0.27905	0.40433	1.45
T ₄		0	5.62	24.56	18.94	336.90	0.27052	0.42600	1.58
T ₅	2.5	500	5.62	25.65	20.03	356.55	0.28614	0.39967	1.40
T 6		1000	5.61	29.08	23.47	418.03	0.33524	0.34667	1.03
T ₇		0	5.61	22.69	17.08	323.49	0.25943	0.49800	1.92
T ₈	5	500	5.61	27.52	21.91	354.94	0.28448	0.41833	1.47
T 9		1000	5.62	28.31	22.69	403.43	0.32410	0.40233	1.24
	PSE		0.02	0.42	0.40	6.22	0.01	0.01	0.03

Data are means of three replicates.

PSE = pooled SE = $\sqrt{MSE/n}$ (where MSE = mean-squared error).

Table 4 showed that there was a significant difference in nutrients discharge through feces. Maximum digestibility values of dry matter (30%), crude protein (70%), crude fat (75%) and gross energy (71%) of guar meal based diet were observed in the test diet

 (T_6) having 2.5% CA and 1000 FTU kg⁻¹ level. The next highest values of dry matter: 23%, crude protein: 66%, crude fat: 73% and gross energy: 67% were observed with 5% CA and 1000 FTU kg⁻¹. It was found that these maximum values were significantly (p<0.0005)

different from the values analyzed for the control diet (dry matter: 18%, crude protein: 47%, crude fat: 60% and gross energy: 40%) as represented in Table 6.

Table 4: Analyzed nutrient composition in *Cirrhinus mrigala* fingerlings feces fed on CA and phytase supplemented guar meal based diets.

Treatments	CA (%)	Phytase level (FTU kg ⁻¹)			Crude fat (%)	Gross energy (kcal g ⁻¹)
T_1		0	86.71	19.23	2.30	2.12
T_2	0	500	82.96	17.66	1.99	1.93
T_3		1000	83.40	17.24	1.84	1.83
T_4		0	84.78	17.89	1.74	1.83
T_5	2.5	500	80.94	14.70	1.64	1.28
T_6		1000	73.29	10.54	1.40	1.01
T_7		0	85.21	18.45	1.84	2.01
T_8	5	500	81.97	14.02	1.65	1.23
T_9		1000	81.96	12.40	1.53	1.13
	PSE		0.80	0.42	0.02	0.04

Data are means of three replicates.

PSE = pooled SE = $\sqrt{MSE/n}$ (where MSE = mean-squared error).

Table 5: Apparent digestibility coefficient (%) of nutrients to Cirrhinus mrigala fingerlings fed on CA and phytase supplemented guar meal based diet.

Treatments	CA (%)	Phytase level (FTU kg ⁻¹)	Dry matter (%)	Crude protein (%)	Crude fat (%)	Gross energy (%)
T_1		0	17.75	47.00	59.97	40.06
T_2	0	500	19.24	49.77	64.26	43.91
T_3		1000	21.69	52.45	67.98	48.23
T_4		0	20.67	50.66	69.61	47.82
T_5	2.5	500	22.83	58.97	71.19	62.75
T_6		1000	30.75	70.29	74.93	70.49
T_7		0	20.15	49.05	67.87	42.46
T_8	5	500	21.80	60.55	70.64	64.12
T_9		1000	23.27	65.73	73.24	67.32
	PSE		0.68	1.19	0.44	1.53

Data are means of three replicates.

PSE = pooled SE = $\sqrt{MSE/n}$ (where MSE = mean-squared error).

Analysis of control and test diets revealed that there was an equal amount of minerals in all the diets but different in feces (Tables 6 and 7). Lowest minerals were noted in feces when fingerlings were fed on 2.5% CA and 1000 FTU kg⁻¹ level based diet. Maximum ADC% values of minerals (Ca: 69%, P: 77%, Na%: 65%, K: 62%, Mg: 54%, Fe: 66%, Cu: 58%, Mn: 70% and Zn 74%) were noted for *C. mrigala* fingerlings when fed on guar meal supplemented with 1000 FTU kg⁻¹ and 2.5% of CA. The next highest values of

mineral digestibility were observed with 1000 FTU kg⁻¹ with 5% of CA. These values found with 2.5% CA and 1000 FTU kg⁻¹ level based diet, were significantly different from control and other test diets as shown in Table 8. Lowest mineral digestibility (Ca: 41%, P: 44%, Na%: 42%, K: 42%, Mg: 29%, Fe: 31%, Cu: 40%, Mn: 43% and Zn 44%) was observed in fish fed on the control diet. From these results, it was noted that 1000 FTU kg⁻¹ and 2.5% of CA supplementation is necessary for maximum fish performance after high

digestibility of nutrients and mineral. The digestibility values at the mentioned levels differed significantly (p<0.05) as compared to the control and other test diets. This revealed that both the above levels of phytase and CA in corresponding feeds were effective to break down the mineral-phytate

complexes and thus increase the availability of nutrients and minerals resulting in increased fish growth performance. A significant interaction was also observed between phytase and CA for improving minerals digestibility.

Table 6: Analyzed minerals (%) composition in diets of *Cirrhinus mrigala* fingerlings fed on CA and phytase supplemented guar meal based diet.

Treatments	CA (%)	Phytase level (FTU kg ⁻¹)	Ca	P	Na	K	Mg	Fe	Cu	Mn	Zn
T_1		0	0.143	1.980	0.784	1.282	0.077	0.072	0.754	0.091	0.071
T ₂	0	500	0.143	1.976	0.773	1.268	0.078	0.071	0.745	0.091	0.073
T ₃		1000	0.143	1.982	0.798	1.278	0.080	0.070	0.754	0.094	0.073
T ₄		0	0.143	1.975	0.795	1.264	0.078	0.072	0.745	0.092	0.072
T ₅	2.5	500	0.142	1.972	0.776	1.270	0.078	0.072	0.748	0.093	0.070
T ₆		1000	0.143	1.989	0.788	1.260	0.078	0.073	0.750	0.094	0.075
T ₇		0	0.142	1.973	0.771	1.278	0.081	0.074	0.751	0.090	0.072
T ₈	5	500	0.142	1.986	0.798	1.266	0.079	0.072	0.754	0.094	0.072
T ₉		1000	0.142	1.979	0.793	1.271	0.079	0.074	0.745	0.095	0.072
	PSE		0.0007	0.0011	0.0015	0.00175	0.00076	0.00082	0.0014	0.0011	0.0013

Data are means of three replicates.

PSE = pooled SE = $\sqrt{MSE/n}$ (where MSE = mean-squared error)

Table 7: Analyzed minerals composition (%) *Cirrhinus mrigala* fingerlings feces fed on CA and phytase supplemented guar meal based diets.

Treatments	CA (%)	Phytase level (FTU kg ⁻¹)	Ca	P	Na	K	Mg	Fe	Cu	Mn	Zn
T_1		0	0.094ª	1.233ª	0.508ª	0.828ª	0.061ª	0.056ª	0.503ª	0.058ª	0.044ª
T_2	0	500	0.085 ^b	0.991 ^b	0.473 ^b	0.753 ^b	0.053 ^b	0.047 ^b	0.414 ^b	0.052 ^b	0.040 ^b
T ₃		1000	0.079^{d}	0.889^{d}	0.408^{d}	0.728°	0.054 ^b	0.043 ^d	0.404^{d}	0.051 ^b	0.037°
T_4		0	0.070e	0.815e	0.390e	0.695 ^d	0.052 ^b	0.051e	0.451e	0.056ª	0.043ª
T_5	2.5	500	0.066 ^f	0.720 ^f	0.366 ^f	0.657e	0.051 ^b	0.037^{f}	0.380^{f}	0.043°	0.027e
T_6		1000	0.048^{i}	0.496^{i}	0.297^{i}	0.527h	0.039e	0.027^{i}	0.340^{i}	0.031e	0.021g
T_7		0	0.083°	0.948°	0.445°	0.756 ^b	0.055 ^b	0.053°	0.468°	0.057ª	0.044ª
T ₈	5	500	0.059g	0.656 ^g	0.347≊	0.626 ^f	0.046°	0.040≊	0.393≊	0.038^{d}	0.031 ^d
T ₉		1000	0.051^{h}	0.540 ^h	0.334^{h}	0.584 ^g	0.043 ^d	0.032^{h}	0.361^{h}	0.037^{d}	0.023 ^f
	P	SE	0.00086	0.00198	0.0023	0.00185	0.00091	0.00115	0.0015	0.00143	0.0012

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

PSE =pooled SE= $\sqrt{MSE/n}$ (where MSE = mean-squared error).

Table 8: ADC% of minerals for *Cirrhinus mrigala* fingerlings fed on CA and phytase supplemented guar meal based diet.

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Treatments	CA (%)	Phytase level (FTU kg ⁻¹)	Ca	P	Na	K	Mg	Fe	Cu	Mn	Zn
T_1		0	40.79^{i}	44.12^{i}	41.91 ⁱ	42.03 ⁱ	29.21 ⁱ	30.60g	40.01 ⁱ	42.80g	44.17 ^f
T_2	0	500	44.99 ^h	53.59h	43.38^{h}	45.00 ^h	36.45 ^h	38.241 ^f	48.360 ^h	47.281 ^f	48.85e
T_3		1000	50.52 ^f	59.74 ^f	54.10 ^f	48.89 ^f	39.16 ^f	45.25°	51.86 ^f	50.836e	54.69 ^d
T_4		0	56.11e	62.92e	55.91e	50.59e	33.52e	36.367 ^f	45.640e	45.831 ^f	46.504 ^f
T ₅	2.5	500	57.78 ^d	66.84 ^d	57.14 ^d	53.03 ^d	40.85 ^d	52.71°	54.16 ^d	58.185 ^d	64.674 ^d
T_6		1000	69.16ª	77.16ª	65.41ª	61.67ª	54.34ª	65.95ª	58.18ª	69.628ª	74.20ª
T_7		0	47.49 ^g	56.82g	48.10 ^g	46.88g	38.75 ^g	35.526 ^f	43.789g	43.429g	45.270 ^f
T ₈	5	500	62.25°	69.76°	60.26°	54.76°	47.04°	48.926 ^d	52.027°	62.773°	60.175°
T ₉		1000	67.72 ^b	75.41 ^b	62.11 ^b	58.62 ^b	51.57b	61.052 ^b	56.650 ^b	65.375 ^b	70.81 ^b
	PSE		0.7333	0.3578	0.5382	0.4556	1.4263	1.5671	0.4950	1.4159	1.5778

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

PSE = pooled SE = $\sqrt{MSE/n}$ (where MSE = mean-squared error).

Discussion

The addition of CA to the diet reduces the pH of the stomach and enhances the phytase activity to break down the phytate complexes (Baruah et al., 2005). In addition to this, the epithelial cell proliferation in the GIT mucosa is also stimulated by CA (Sakata et al., 1995) and thus the digestibility of nutrients increases. In the current study the maximum values in term of growth, nutrients and mineral digestibility for C. mrigala fingerlings fed on guar meal based test diets were noted at 1000 FTU kg⁻¹ with 2.5% CA supplemented diet significantly different from control and other test diets. Baruah et al. (2007a) found maximum growth performance at 3% CA and 500 FTU kg⁻¹. Similarly, Zhu et al. (2014) reported that when 2 g of CA was combined with 500 FTU kg ¹ level of phytase, juveniles of yellow cat fish showed maximum growth. Similar results of higher growth were reported by Hussain et al. (2015), but the level was a little different from the present results. They reported that supplementation of 5 % CA and 500 FTU Kg⁻¹, resulted in the maximum growth of C. mrigala fingerlings fed corn gluten meal (30%) based diet. In literature, it was found that for fish feeds the optimal levels of CA are 2.5% to 5 % (Khajepour et al., 2012a; Baruah et al., 2007b) and optimal levels of supplementation phytase between 250 and 1500 FTUkg⁻¹ levels (Cao et al., 2007). These variations in findings depend upon the sources for origin of phytases, experimental fish species, diet making technology and studied response parameters.

Results of the present study are also supported by Yu and Wang (2000) who reported that addition of phytase 1000 FTUkg⁻¹ enhanced average weight gain of fish by 25%. Similar results were found by Nwanna and Schwarz (2008) in which they noted an increase in growth performance at 750 and 1000 FTU kg⁻¹ levels of phytase supplementation in diets of *Cyprinus*

carpio. However, Hussain et al. (2011) found that phytase supplementation with plant-meal based diet at 750 FTU kg⁻¹ level is optimal for highest growth performance of L. rohita fingerlings. In other studies, Hossain et al. (2007) and Sarker et al (2007) in red sea bream, P. major, Pandey and Satoh (2008) in rainbow trout, O. mykiss and Sarker et al. (2012b) in yellow tail found that 1% level of CA is optimum for maximum growth performance. Variations have been seen in the results of different researchers related to unpredictable factors such as water quality parameters, feed ingredients and citric acid sources (Liu et al., 2013).

The dosage of CA and phytase supplementation differs in reported studies for enhancing nutrient digestibility in fish. According to the present results, it was found that supplementation of CA (2.5%) and phytase (1000 FTU kg⁻¹) enhances fish growth as compared to the control diet. Nearly similar to our results, Baruah et al. (2005) found the maximum crude protein digestibility at 3% CA and 500 FTU kg⁻¹ phytase. Whereas, Saeed observed (2012)highest protein digestibility in L. rohita fingerlings fed on 5% CA with 750 FTU kg⁻¹ level. The findings of the current study are also supported by Ashraf and Goda (2007). They observed higher crude fat digestibility at 1000 FTU kg⁻¹ level after which it decreased. Saeed (2012) noted the highest crude fat digestibility at 5% CA and 750 FTU kg-1 level of phytase supplementation. Contrary to the current results, Khajepour et al. (2011) reported that addition of CA in

the fish diet reduced the digestibility of nutrients. Phromkunthong et al. (2010) reported an improvement in nutrient digestibility in Common carp fed plant meal based diet supplemented with CA (0.22%) and phytase (750 FTU kg⁻¹). Dry matter, crude protein, crude fat and gross energy digestibility were higher in L. rohita fed on canola meal based diets supplemented with 3% CA and 500 FTU kg⁻¹ (Arshad, 2013) and 1000 FTU kg⁻¹ level of phytase (Igbal, 2012). On the contrary, Baruah et al. (2007b) did not observe any effect on crude protein and dry matter digestibility in response to the interaction of phytase and CA.

CA enhances the mineral availability for fish when fed on plant meal and mitigate the inhibitory effect of antinutritional component (phytate) on mineral digestibility (Khajepour and Hosseini, 2012b). The results of the current study proved the synergetic effects of CA and phytase because the availability of minerals was significantly improved after acidification of phytase supplemented diets. The digestibility values of Ca, P, Na, K, Mg, Cu, Mn, Zn, and Fe were highest at 2.5% CA and 1000 FTU kg⁻¹ in the case of guar meal based test diets. Similarly, the synergetic effect of CA and phytase on digestibility of minerals was noted by Baruah et al. (2007a). They reported a significant interaction effect between CA (3%) and phytase (500 FTU kg⁻¹) on the absorption of P, Na, K, Mg, Mn, and Fe in Labeo rohita fingerlings fed on a plant based diet. reported Saeed (2012)maximum digestibility values of minerals at 5%

CA and 750 FTU kg⁻¹ level. Among the minerals, their synergistic effect has a special influence on P availability, (Phromkunthong et al., 2010) and retention in fish (Baruah et al., 2005). On the contrary, non-significant interactions were observed between phytase and CA on the mineral utilization of juvenile yellow catfish (Zhu et al., 2014). Soybean meal treated with phytase and CA had no effects on the calcium and phosphorus of muscle, scute and serum in beluga Huso huso (Khajepour et al., 2011). So the results of the current work are supported by the above studies and variations in some findings might be associated with a difference in diet composition, fish species, and rearing conditions.

In conclusion, this study provided evidence that acidification of phytase treated guar meal-based diets increased the growth performance of C. mrigala fingerlings by improving nutrient digestibility and mineral availability, as well as reduced the nutrients discharge into the water. It also showed a great interaction between CA and phytase regarding increase mineral digestibility in fish when fed on a plant meal based diet. The optimum levels of CA and phytase supplementation for guar meal are 2.5% and 1000 FTU kg⁻¹.

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