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Full Length Article

Effect of Citric Acid and Phytase on Growth Performance and Mineralization of *Labeo rohita* Juveniles Fed Soybean Meal Based Diet

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Abstract

Present experiment was conducted to investigate the effects of phytase, citric acid and their interaction on growth, muscle proximate composition and mineralization of whole body and bones in *Labeo rohita* juveniles. For this trial, 405 juveniles were used in a 3^2 factorial arrangement (0, 1.5 and 3% citric acid and 0, 750 and 1000 FTU/kg phytase) under completely randomized design. Triplicate groups of 15 fish per experimental units were fed experimental diets for 8 weeks. Fish fed citric acid and phytase supplemented diet showed improved ($p<0.05$) growth and body proximate of *L. rohita* either supplemented individually or mutually. Citric acid addition caused a significant ($p<0.05$) increment in the minerals deposition in the whole body and bones of juveniles. Similarly, phytase supplementation also improved ($p<0.05$) the mineralization in juveniles. Furthermore, a significant ($p<0.05$) interaction between both the supplements was observed to improve the minerals contents in the body and bones of fish. On the basis of these results, it is concluded that citric acid and phytase are very effective supplements to increase the bioavailability of minerals from soybean meal based diet.

Keywords: Citric acid; Fish; Phytase; Bone mineralization

Introduction

Fishmeal is considered a healthier source of nutrients as it has appropriate amount of essential fatty acids and amino acids. Besides this, it is highly palatable and also provides highly digestible energy (Tacon, 1993). Several factors hinder the use of fishmeal for sustainable farming like the rising demand, unpredictable availability, high cost, static level of production and restricted supply. These elements make it pre-requisite to seek for novel alternative sources of proteins of plants and animals origin. Plant origin ingredients make a major part of feed for fishes like carps including *Labeo rohita* (Higgs *et al.*, 1995; New and Wijkstrom, 2002; Baruah, 2004). However, major drawback in the use of plant proteins are certain factors, such as phytate, which reduce the bioavailability of nutrients to fish (Francis *et al.*, 2001).

Phytate is present in all legumes, cereals, nuts and oil seeds and has about 60 − 90% of total phosphorus as phosphate present in plants (Raboy, 2003). Phytate has also strong capability to reduce the bioavailability of other minerals to fish by its direct or indirect interaction. Being negatively charged, phytate makes complexes with many cations like calcium, potassium, sodium, magnesium, manganese, iron, copper and zinc (Leiner, 1994). Indirectly, Ca-phytate complexes enhance the co-precipitate formation of zinc and many other trace minerals by the process of chelation, rendering them unavailable to fish (O`Dell, 1962). It prevents the assimilation of starch and proteins in the digestive tract as it has affinity to bind with them (Noureddini and Dang, 2008). Hence, presence of phytate is the main predicament to use the plant protein sources in fish feed formulation as it limited the availability of minerals. Furthermore, phytate bounded undigested phosphorus is excreted through feaces in the ponds. Microorganisms degrade the phytate-P complex which leads to the algal blooms and consequently lead to deficiency of oxygen in water (Kaur *et al.*, 2007).

Phytases are phosphatases which yield free inorganic phosphorus as intermediates by sequentially cleaving the groups of orthophosphate from the inositol ring of phytate (Li *et al.*, 1997). Now a days, these phytases are being supplemented in the diet of fish to hydrolyze the phytate. Improved mineralization in response to phytase supplementation have been reported in yellow catfish (Zhu *et al.*, 2014), African catfish (Nwanna *et al.*, 2005), Nile tilapia (Liebert and Portz, 2005) sea baas (Naret, 2013) and *Salmo salar* (Denstadli *et al.*, 2007).

Another approach which is being applied in fish nutrition to break the phytate is the supplementation of

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organic acids to the diet. Supplementary organic acids cause low intestinal pH, which enhances the solubility of phytate-minerals complexes resulting in improved absorption of released minerals (Jongbloed, 1987). Besides having impact on pH, organic acids act as chelating agents and bind numerous cations, which lead to enhanced absorption of minerals in intestine (Ravindran and Kornegay, 1993). Among these organic acids, citric acid (CA), due to its unique flavor and high buffering capacity, has been extensively used for diet acidification (Hossain *et al*., 2007). Its efficacy to dephosphorylate the phytate *in vitro* has also been reported (Zyla *et al.,* 1995). Citric acid supplementation resulted in improved P contents in the scute of *Huso huso* fed on plant meal based diet (Khajepour and Hosseini, 2011; 2012). Baruah *et al.* (2005) also reported improved bone mineralization in *L. rohita* juveniles by supplementing citric acid in soybean meal based diet.

Since citric acid lowers the gastric pH, its supplementation can also favor the action of phytase as it works optimally at pH 5.0-5.5 and 2.5 (Simons *et al.,* 1990). In addition, dietary acidification may lower the gastric emptying speed (Mayer, 1994) which provides more time for nutrient absorption and also enhances the action of phytase. Improved bone P contents of common carp as a result of phytase and citric acid interaction has been reported by Phromkunthong *et al.* (2010). Hence, it is hypothesized that supplementation of citric acid and phytase simultaneously may cause synergistic effects to improve growth performance, body proximate composition and mineralization.

Materials and Methods

Preparation of Experimental Diets

Present experiment was designed to investigate the main and interaction effects of citric acid and phytase on growth performance, body proximate composition minerals utilization in *L. rohita* juveniles. Nine isonitrogenous (28.40), isocaloric (3.85) and isolipidic (7.52) experimental diets were prepared having 12% fishmeal and 56% soybean meal as protein source. Fish oil was added as lipid and energy source. Citric acid was added at the level of 0, 1.5 and 3% while phytase was included at 0, 750 and 1000 FTU/kg level in the experimental diets in 3^2 factorial arrangement under completely randomized design in triplicates (Table 1).

For the formulation of experimental diets, dry ingredients were grounded and screened (0.05 mm) in cereal grinding machine (FFC-45, JIMO, China). Citric acid, minerals mixture, vitamin premix and fish oil were added to ground ingredients and mixed electrically. Appropriate amount of water was added to make dough which was further processed to make pellets (3 mm) through hand pelletizer. Pellets were sprayed with three concentrations of liquid phytase (Phyzme®xP 10000 FTU/g, Damisco Animal Nutrition. Fin-65 101 Vaasa Finland), such that 1 mL liquid phytase solution (2 g powder phytse/1 L water) had 20 FTU in it (Robinson *et al.,* 2002). Pellets were blow dried up to 10% moisture, sealed in vacuum packed bags and stored at -20°C throughout the feeding trial. Proximate composition of diet is also given in Table 1. Standard methods of AOAC (1995) were adopted for determination of proximate composition of ingredients, diets and whole body; moisture by drying sample at 105°C to a constant weight, crude protein by determination of N on micro Kjeldahl apparatus after acid digestion and N×6.25 formula, crude fat by ether extraction through soxtec HT2 1045 system, while adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline. USA) was used for estimation of gross energy of samples.

Experimental Fish and Feeding Trial

Healthy juveniles of *L. rohita* (average weight 6.93±0.30) were obtained from Government Fish Seed Hatchery, Faisalabad, Pakistan. Before initiation of feeding trial, fish were bathed in 5 g/L NaCl solution and allowed to acclimatize in V shaped experimental tanks. During acclamation fish were provided basal diet once a day with continuous aeration. Fifteen fish were randomly stocked in each triplicate of tanks for feeding trial. Filtered fresh water and continuous aeration was provided to each experimental tank for optimum amount of dissolved oxygen (5.8-7.3 mg/L) for fish culture. Water temperature and pH were set at 24.9 −28.7°C and 7.4−8.6, respectively throughout feeding experiment. Fish were fed twice daily up to apparent satiation 6 days a week and uneaten diet was siphoned after 2 h of feeding. Feeding experiment lasted for 8 weeks.

Sampling and Chemical Analysis

At the termination of feeding experiment, fish were starved for 24 h, dipped in 3000 mg/L solution of clove oil for 40-60 s to anesthetize (Khajepour *et al.,* 2012) and sacrificed by a sharp blow on head. Five fish from each replicate was minced and dried at 60°C for whole body proximate and minerals analysis. For bone minerals analysis 5 whole fish were boiled for 20 min in water until the flesh was easily stripped off from bones. Soft tissues were separated from vertebrae by slight brushing. Vertebrae were then rinsed with distilled water and oven dried at 110°C for 2 h, defatted for seven hours with anhydrous ethyl ether, pulverised in mortar and pestle, again dried and finally weighed. Minerals estimation was processed by acid digestion following AOAC, (1995). Samples (whole body and bones) were digested in 2:1 boiling nitric acid and perchloric acid mixture. After appropriate dilution Ca, Mg, Mn, Zn, Cu and Fe contents were estimated on atomic absorption spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) while Na and K were estimated on flame photometer (Jenway PFP-7, UK). After molybdate reagent

oxidation, P was estimated with the help of UV-VIS spectrophotometer (U-2001, Hitachi) at 750 nm absorbance.

Statistical Analysis

All data was subjected to two way analysis of variance. When significant differences occurred, Tukey's Honestly Significant Difference Test for comparison of means at 5% significance level was applied (Snedecor and Conhran, 1991). All statistical analyses were done using CoStat computer package (Version 6.303, PMB 320, Monterey, CA, 93940 USA).

Results

Growth Performance

Growth performance of *L. rohita* fingerlings in response to dietary phytase and citric acid supplementation is shown in Table 2. Supplemental phytase significantly ($p<0.05$) increased the final weight (FW), weight gain (WG), weight gain percent (WG%) and specific growth rate (SGR) with maximum gain at 1000 FTU/kg phytase level. These growth parameters were also improved ($p<0.05$) by the addition of citric acid in the diet. Moreover, the interaction of phytase and citric acid exerted positive ($p<0.05$) effect on the performance of juveniles.

Whole Body Proximate

Effects of dietary addition of phytase, citric acid and their interaction on proximate body composition of fingerlings are summarized in Table 3. Improved ($p<0.05$) dry matter, crude protein, crude fat and ash contents were observed in diets supplemented with phytase and citric acid as compare to control group. Also, positive interaction ($p<0.05$) between phytase and citric acid was found for these nutritional attributes.

Whole Body Mineralization

Minerals contents of whole body of rohu were found significantly ($p<0.05$) affected by citric acid and phytase supplementations (Table 4). Different levels of phytase behaved differently and maximum minerals deposition in the juvenile's body was recorded at its highest level (1000 FTU/kg) of supplementation. Citric acid supplementation also significantly ($p<0.05$) enhanced the whole body mineralization of juveniles. Furthermore, both of the supplements interacted with each other significantly and maximum deposition of these minerals was observed at 3% citric acid and 1000 FTU/kg phytase level. However, this synergism was not recorded for P, Mg and Zn.

Bones Mineralization

Data of main effects of phytase and citric acid and their interaction is shown in Table 5. Main effect data of phytase supplementation showed improved ($p<0.05$) minerals contents in bones of juveniles. Similarly, citric acid addition also caused variations in the bone minerals contents of fish. Maximum mineral deposition in bones was recorded in 3% citric acid containing diet as compare to other acidified diets. Nevertheless, analysis of variance showed a non-significant interaction between citric acid and phytase for bone mineralization except Cu.

Discussion

In this study, supplementation of dietary phytase had resulted in enhanced ($p<0.05$) growth performance of fingerlings. This increased growth response may be attributed to increased availability of nutrients and minerals due to enzymatic breakdown of phytate-nutrient complexes. Increased growth in our study is confirmatory to the observations made for various fish species including rohu (Hussain *et al.,* 2011), common carp (Phromkunthong *et al.,* 2010) gibel carp (Liu *et al.,* 2012) and tilapia (Trichet *et al.,* 2014).

Citric acid supplementation, in the present study, also resulted in enhanced growth performance of fingerlings in soybean meal based diet. Citric acid may lowered the intestinal pH, which favors the phytate-nutrient complex solubility and nutrients absorption from gastrointestinal tract resulting in improved growth performance of fish (Cross *et al.,* 1990). Similarly, addition of lower levels of citric acid (1–3 g/kg) had also resulted in increased weight gain in tilapia (Ng *et al.,* 2009; Koh *et al.,* 2014).

Results of the present study demonstrated synergistic effect between phytase and citric acid for improving growth performance of fingerlings. Present positive synergistic interaction between these two supplements to improve growth performance is in accordance to Baruah *et al.* (2005; 2007b) for same fish species. Citric acid may have provided the optimum conditions to the phytase by lowering the intestinal pH, which led to positive interaction among both supplements.

In the present study, addition of phytase in diets resulted in significant ($p<0.05$) improvement in whole body dry matter, crude protein and crude ash while crude fat contents of *L. rohita* fingerlings were reduced. This may be due to phytate hydrolyzing tendency of dietary phytase, which may release the chelated nutrients into the digestive tract of fish (Tudkaew *et al.,* 2008). In contrast to our findings, non-significant effect of phytase on dry matter contents of Atlantic salmon (Carter and Sajjadi, 2011) has been reported. In agreement to our results, Sardar *et al.* (2007) reported positive effect of phytase treatment on whole body crude protein contents of *Cyprinus carpio*. A significant decrease in crude fat and increase in crude ash contents of *Salmo salar* against phytase supplementation was observed by Denstadli *et al.* (2007).

Table 1: Ingredient composition of experimental diets

Citric acid (%)	0			1.5			3		
Phytase (FTU/kg)	0	750	1000	0	750	1000	0	750	1000
Ingredients (%)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9
Soybean meal	56	56	56	56	56	56	56	56	56
Fish meal	12	12	12	12	12	12	12	12	12
Rice polish	12	12	12	12	12	12	12	12	12
Wheat flour	10	10	10	10	10	10	10	10	10
Fish oil	6	6	6	6	6	6	6	6	6
Vitamin premix	1	1	1	1	1	1	1	1	1
Mineral mixture	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
Total	100	100	100	100	100	100	100	100	100
Proximate composition									
Dry matter (%)	93.03	93.27	93.36	93.2	93.25	93.54	93.31	93.48	93.67
Crude protein (%)	28.5	28.35	28.75	28.3	28.19	27.9	28.58	28.75	28.25
Crude fat (%)	7.57	7.43	7.57	7.51	7.54	7.53	7.54	7.21	7.79
Gross energy (kcal/g)	3.91	3.85	3.87	3.87	3.83	3.74	3.82	3.95	3.83

Table 2: Citric acid, phytase and their interaction with growth performance of *L. rohita* juveniles fed soybean meal based diet

Citric acid (%)	0			1.5			3			PSE	Analysis of Variance		
Test Diets	T1	T2	T3	T4	T5	T6	T7	T8	T9		Citric acid	Phytase	Citric acid×Phytase
Initial Weight (g)	13.36	13.56	13.56	13.62	13.22	13.42	13.21	13.6	13.6				
Final Weight (g)	19.65^{c}	20.89^{bc}	21.55^{bc}	21.21^{bc}	23.53^{b}	24.53^{ab}	22.13^{bc}	25.2^{ab}	26.68^{a}	1.58	p<0.05	p<0.05	p>0.05
Weight Gain (g)	6.29^{f}	7.33^{e}	7.99^{de}	7.59^{e}	10.31^{c}	11.11^{bc}	8.93^{d}	11.6^{b}	13.08^{a}	0.54	p<0.05	p<0.05	p>0.05
Weight Gain %	47.05^{h}	54.05^{g}	58.89^{f}	55.71^{g}	78.01^{d}	82.81^{c}	67.58^{e}	85.29^{b}	96.19^{a}	1.11	p<0.05	p<0.05	p<0.05
Specific Growth Rate	0.43^{h}	0.48^{g}	0.52^{f}	0.49^{g}	0.64^{d}	0.67^{c}	0.57^{e}	0.69^{b}	0.75^{a}	0.01	p<0.05	p<0.05	p<0.05

Table 3: Citric acid, phytase and their interaction with whole body proximate of *L. rohita* juveniles fed soybean meal based diet

Citric acid (%)	0			1.5			3						
Phytase (FTU/kg)	0	750	1000	0	750	1000	0	750	1000		Analysis of Variance		
Test Diets	T1	T2	T3	T4	T5	T6	T7	T8	T9	PSE	Citric acid	Phytase	Citric acid × Phytase
Dry Matter (g/kg)	277.05^{h}	292.75^{g}	296.45^{e}	296.15^{f}	308.5^{c}	304.9^{d}	298.55^{e}	312.7^{b}	316.5^{a}	1.00	p<0.05	p<0.05	p<0.05
Crude Protein (g/kg)	192.55^{d}	202.65^{c}	204.3^{c}	205.2^{c}	211.5^{b}	214.1^{b}	204.85^{c}	215.6^{ab}	217.9^{a}	1.25	p<0.05	p<0.05	p>0.05
Crude Fat (g/kg)	67.05^{a}	55.5^{b}	52.35^{c}	$53.9b^{c}$	46.8^{d}	46.35^{d}	54.3^{bc}	43.5^{e}	42.6^{f}	0.94	p<0.05	p<0.05	p>0.05
Crude Ash (g/kg)	44.6^{h}	50.85^{g}	55.45^{e}	55.6^{e}	59.95^{d}	62.55^{c}	52.95^{f}	64.45^{b}	66.5^{a}	0.81	p<0.05	p<0.05	p<0.05

In the present study, citric acid appeared to improve the whole body proximate due to its phytate hydrolyzing capabilities. Hossain *et al. (*2007) reported increased crude protein contents, while reduced crude fat, crude ash and moisture contents in the fish body in organic acid supplemented red sea bream.

A significant interaction between phytase and citric acid was observed, in the present study, for dry matter and crude ash of *L. rohita.* Supplementation of citric acid might decrease the pH of gastro-intestinal tract (Erdman, 1979) as well as reduced the rate of gastric emptying (Mayer, 1994), both of these actions provided more favorable environment for phytase to act.

Whole body and bone mineralization, in this experiment, was enhanced (p<0.05) in fish fed the phytase treated diet. Improved mineralization in the present study might be due to phytate hydrolysis resulting in release of bound minerals, which led to increased body and bone mineral contents. Similar to present results, increased (p<0.05) P contents in whole body of Atlantic salmon (Carter and Sajjadi, 2011) and *Cypriuns carpio* (Sardar *et al.,* 2007) were also observed in response to phytase supplementation. Liu *et al.* (2014) reported improved whole body Mg and Zn contents in fish fed diet having phytase supplementation. Improved P, Ca, Mg and Zn contents were observed in the bones of *Salmo salar* (Denstadli *et al.* 2007) and red sea bream (Laining *et al.,* 2012) having phytase treated diets.

In the present study, citric acid addition also resulted in enhanced whole body and bone mineralization of rohu juveniles which indicates the hydrolysis of phytate. Similar to our study increased (p<0.05) whole body P, Ca, K, Cu, Mn and Fe contents were observed in *Pagrus major* fed on 3% citric acid supplemented diet (Laining *et al.,* 2012).

Table 4: Citric acid, phytase and their interaction with whole body mineralization of *L. rohita* juveniles fed soybean meal based diet

Citric acid (%)	0			1.5			3						
Phytase (FTU/kg)	0	750	1000	0	750	1000	0	750	1000	Analysis of Variance			
Test Diets	T1	T2	T3	T4	T5	T6	T7	T8	T9	PSE	Citric acid	Phytase	Citric acid × Phytase
P (%)	0.95^{f}	1.60^{e}	1.65^{e}	1.70^{de}	2.55^{c}	2.75^{b}	1.80^{d}	2.60^{c}	2.95^{a}	0.06	p<0.05	p<0.05	p>0.05
Ca (%)	0.88^{c}	0.96^{b}	0.95^{b}	0.96^{b}	1.55^{a}	1.85^{a}	0.99^{b}	1.65^{a}	1.80^{a}	0.02	p<0.05	p<0.05	p<0.05
Mg (%)	0.85^{g}	1.55^{f}	1.75^{de}	1.70^{e}	2.40^{c}	2.45^{c}	1.85^{d}	2.65^{b}	2.90^{a}	0.06	p<0.05	p<0.05	p>0.05
Na (mg/kg)	3.30^{f}	3.85^{e}	3.85^{e}	4.05^{de}	5.50^{c}	5.65^{c}	4.20^{d}	5.95^{b}	6.40^{a}	0.11	p<0.05	p<0.05	p<0.05
K (mg/kg)	4.40^{f}	4.75^{e}	5.05^{d}	5.00^{d}	6.45^{c}	6.75^{b}	4.95^{d}	6.80^{b}	7.20^{a}	0.08	p<0.05	p<0.05	p<0.05
Mn (ug/g)	5.90^{h}	6.35^{g}	6.90^{e}	6.65^{f}	8.40^{d}	8.70^{c}	6.85^{e}	9.10^{b}	9.35^{a}	0.08	p<0.05	p<0.05	p<0.05
Fe (ug/g)	28.00^{f}	34.00^{e}	37.50^{d}	37.00^{d}	52.00^{c}	55.00^{b}	36.5^{d}	56.5^{b}	62.50^{a}	0.86	p<0.05	p<0.05	p<0.05
Cu (ug/g)	2.20^{e}	2.80^{d}	3.10^{c}	2.65^{d}	4.05^{b}	4.35^{a}	2.70^{d}	4.40^{a}	4.50^{a}	0.11	p<0.05	p<0.05	p<0.05
Zn (ug/g)	13.01^{f}	19.5^{e}	19.5^{e}	21.31^{d}	26.5^{c}	29.5^{b}	22.11^{d}	30.12^{a}	31.5^{a}	1.22	p<0.05	p<0.05	p>0.05

Table 5: Citric acid, phytase and their interaction with bones mineralization of *L. rohita* juveniles fed soybean meal based diet

Citric acid (%)	0			1.5			3						
Phytase (FTU/kg)	0	750	1000	0	750	1000	0	750	1000	Analysis of Variance			
Test Diets	T1	T2	T3	T4	T5	T6	T7	T8	T9	PSE	Citric acid	Phytase	Citric acid × Phytase
P (%)	13.30^{b}	13.85^{ab}	13.95^{ab}	13.85^{ab}	14.60^{a}	14.85^{a}	13.60^{ab}	14.70^{a}	14.85^{a}	0.07	p<0.05	p<0.05	p>0.05
Ca (%)	18.85^{g}	23.10^{f}	25.70^{e}	24.05^{f}	27.45^{cd}	26.40^{de}	28.70^{c}	31.05^{b}	34.20^{a}	0.64	p<0.05	p<0.05	p>0.05
Mg (%)	0.51^{e}	0.64^{d}	0.66^{cd}	0.69^{c}	0.84^{b}	0.89^{a}	0.46^{f}	0.86^{ab}	0.91^{a}	0.02	p<0.05	p<0.05	p>0.05
Na (mg/kg)	2.20^{e}	2.40^{de}	2.95^{c}	2.50^{d}	3.45^{b}	3.50^{b}	3.05^{c}	4.00^{a}	4.10^{a}	0.12	p<0.05	p<0.05	p>0.05
K (mg/kg)	0.21^{f}	0.35^{e}	0.35^{e}	0.40^{d}	0.41^{d}	0.43^{c}	0.46^{b}	0.55^{a}	0.56^{a}	0.61	p<0.05	p<0.05	p<0.05
Mn (ug/g)	38.50^{d}	44.05^{c}	43.50^{c}	43.05^{c}	45.70^{b}	47.15^{a}	44.00^{c}	48.00^{a}	47.90^{a}	0.52	p<0.05	p<0.05	p>0.05
Fe (ug/g)	24.0^{f}	26.95^{de}	28.15^{cd}	26.25^{e}	29.30^{bc}	30.00^{b}	28.35^{cd}	31.85^{a}	33.0^{a}	0.64	p<0.05	p<0.05	p>0.05
Cu (ug/g)	14.05^{e}	14.75^{de}	14.90^{de}	15.00^{d}	18.10^{c}	17.50^{c}	14.20^{de}	19.05^{b}	19.95^{a}	0.4	p<0.05	p<0.05	p<0.05
Zn (ug/g)	135.10^{g}	140.00^{f}	141.50^{ef}	142.60^{e}	151.45^{bc}	150.65^{c}	144.20^{d}	152.55^{ab}	153.75^{a}	0.71	p<0.05	p<0.05	p>0.05

Pandey and Satoh (2008) recorded improved P, Ca and Zn contents in the bones of rainbow trout fed diet supplemented with citric acid.

Addition of citric acid in phytase treated diets resulted in enhanced whole body and bone mineralization of rohu juveniles synergistically. Citric acid might had provided optimum conditions to phytase, which lead to significant interaction among both the supplements. Other studies also reported significant interaction between citric acid and phytase to enhance the concentrations of P, Ca, Mg, Na, K, Mn, Fe, Cu and Zn in the body (Baruah *et al.* 2007a) and Ca, P, K and Mn contents in bones of *L. rohita* juveniles.

Conclusion

Phytase and citric acid positively affected the growth performance, meat quality and body mineralization in rohu fingerlings. Moreover, both the supplements interacted significantly with each other for these parameters.

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