Mineral digestibility of *Labeo rohita* fingerlings fed on cottonseed meal based diets supplemented with citric acid and phytase enzyme

Syed Makhdoom Hussain*, Shahtaj Ahmad, Muhammad Mudassar Shahzad, Muhammad Zubair Ul Hassan Arslan, Danish Riaz, Nisar Ahmad, Sadia Tabassum, Abdul Wahab Ahmad

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

**Key words:** Citric acid, Phytase, Cottonseed meal, *Labeo rohita*, Mineral digestibility.

[url](http://dx.doi.org/10.12692/ijb/8.2.25-35) Article published on February 20, 2016

**Abstract**

Present study was conducted to investigate the synergistic effects of citric acid and phytase enzyme on mineral digestibility of *Labeo rohita* fingerlings fed on cottonseed meal based diets. Nine isonitrogenous and isoenergetic test diets were formed by supplementation of different graded levels (0, 400 and 800 FTU kg⁻¹) of phytase and CA (0%, 2% and 4%). Fingerlings were fed at the rate of 5% of live wet weight. Feces were collected from fecal collecting tube. Chromic oxide was added in the feed at 1% as indigestible marker. Highest digestibility values (%) of Ca, P, Na, K, Mg, Cu, Mn, Zn and Fe were observed at 400 FTU kg⁻¹ level with 4% citric acid and these values differed significantly (p<0.05) from the all other test diets. From the present results it was concluded that 4% of citric acid with 400 FTU kg⁻¹ level is sufficient to release chelated minerals to improve overall performance of *Labeo rohita* fingerlings.

*Corresponding Author:* Syed Makhdoom Hussain  drmakhdoom90@gmail.com
Introduction

Pakistan has both inland and marine water resources which can play a significant role in the development of different nutritionally and economically important fish species (Hussain et al., 2011). Labeo rohita commonly known as rohu, is one of the major carp species found in riverine system of South Asia and is also being cultured on a large scale in Pakistan (Abid and Ahmed, 2009; Hussain et al., 2011a). Aquaculture industry is rapidly flourishing all over the world due to its high nutritional value but to maintain its high production is a great challenge for the researchers (Vandenberg et al., 2011). Aquaculture industry is developing more efficiently than any other food producing sectors. However, economic factors such as cost of fish feed are restraining its development (Yildirim et al., 2014). Feed primarily accounts for 50 to 60% of total cost in fish culture (Essa et al., 2004). The main objective for most fish farmers is to produce high quality fish feed at low cost. Fish meal is being used by aqua feed industry as a potential source of essential minerals, nutrients, amino acids, fatty acids and other growth factors (Zhou et al., 2004). But increasing fishmeal prices and restricted supply motivated the researchers to identify the cost effective alternatives of fishmeal (Barnes et al., 2012; Lech and Reigh, 2012; Dedek et al., 2013; Shapawi et al., 2013). Plant proteins, which are usually considered as having low cost than fish meal, have been utilized as a substitute in diets for fresh water fishes (Enterrina et al., 2011; Hussain et al., 2014a; Hussain et al., 2015; Wang et al., 2015). Plant by-products are considered as best alternative protein and energy sources for fish growth (Gatlin et al., 2007; Hussain et al., 2011b; Hussain et al., 2011c) and for the development of cost effective and environment friendly aqua feeds (Cheng and Hardy, 2002; Hussain et al., 2011b).

However, these plant protein sources have anti-nutritional factors such as phytate which make their use limited in fish feed formulation (Baruah et al., 2004). Phytic acid or phytate is a major storage form of phosphorus (P) and has up to 80% of total P present in plant seeds, which is practically not available to agastric or mono-gastric fish species (NRC, 1993). Due to negative charge of phosphate groups present in phytate, it chelates with many cations such as magnesium (Mg), calcium (Ca), copper (Cu), iron (Fe) and zinc (Zn) and form insoluble phytate-mineral complexes (Erdman, 1979). Other than minerals, it also chelates with amino acids, fatty acids and proteins and makes them unavailable to fish. It inhibits the activities of digestive enzymes including lipases, amylases and proteases. Hence, to make plant based fish diets successful, it is compulsory to hydrolyse the phytate to release the chelated nutrients and minerals available to fish (Caipang et al., 2011; Morales et al., 2013). Various efforts have been made to release phosphorous and other chelated minerals from phytate but the most excellent results were obtained by enzymatic hydrolysis of phytate through phytase supplementation (Silva et al., 2005). Phytase, an enzyme is being used as a supplement in plant based diets to hydrolyze the phytate complexes (Cao et al., 2007; Lim and Lee, 2009). Vielma et al. (2004) reported that phytase supplementation increased the concentration of Ca, Mg, Mn and Zn in bone, plasma and the whole body of rainbow trout. Phytase shows its optimum activity at quite low pH in between 2.5 to 5.5 (Cao et al., 2007). But the pH of digestive tract of a gastric or mono-gastric animal is slightly acidic to alkaline for example the pH of common carps digestive system range is 6.5 to 8.4 (Ji, 1999). Organic acids like citric acid decreases the pH of digestive tract of fish when it is incorporated in feed and build up suitable conditions for the phytase enzymes to work efficiently (Sugiura et al., 2001; Baruah et al., 2007; Shah et al., 2015). Reduced intestinal pH increases the solubility of P from phytate and improves P absorption in the small intestine (Khajepour et al., 2012).

Improvement of feed efficiency in Common carp was reported when citric acid and phytase are added in feed (Phromkunthong et al., 2010). Citric acid and phytase showed significant positive interaction for mineral (Zn, Na, P, K, Mn, Mg, Fe, Cu, Ca, and N) absorption, their contents in and whole body (Baruah
et al., 2007) and their apparent digestibility in Labeo rohita fingerlings (Saeed, 2012). Among the minerals, their synergistic effect has special influence on P availability, digestibility and retention in fish (Baruah et al., 2005; Phromkunthong et al., 2010).

Cottonseed meal is the third largest oilseed meal product in all over the world after soybean meal and canola meal (Lee et al., 2006). It is high in protein and generally less expensive per unit of protein than soybean meal (Li and Robinson, 2006). It is an excellent source of plant protein that has long been used in the feeds of terrestrial and aquatic animals (Gatlin III et al., 2007; Lim and Lee, 2008). Cottonseed meal has a plenty of essential amino acids, higher than that of fishmeal and soybean meal (EI-Saidy et al., 2004). It has also been successfully used in various fish species such as parrot fish (Lim and Lee, 2009), tilapia (Mbahinzireki et al., 2001), olive flounder (Pham et al., 2008), sunshine bass (Rawles and Gatlin, 2000) and rainbow trout (Cheng and Hardy, 2002). However there is lack of information about the formulation of artificial feeds from plant proteins for commercially important stomach-less fish species including Labeo rohita (Iqbal et al., 2014).

The objective of present study was to investigate synergistic effects of citric acid and phytase supplementation on mineral digestibility in diets of Labeo rohita fingerlings fed cottonseed meal based diet. Findings from present study will also be helpful in the development of cost effective and environment friendly fish feed.

Materials and methods
Present study was conducted in the Fish Nutrition Laboratory, Department of Zoology, Government College University Faisalabad.

Fish and experimental conditions
Labeo rohita fingerlings were obtained from Government Fish Seed Hatchery, Faisalabad and kept in V-shaped tanks (specially designed for the collection of feces) having 70 L water, for two weeks to acclimatize them to the experimental conditions. Before start of the feeding trial, L. rohita fingerlings were treated with NaCl (5g L⁻¹) to make sure they are free from ectoparasites and also to prevent further fungal infections (Rowland and Ingram, 1991). During this acclimatization period, L. rohita fingerlings were fed basal diet once daily to apparent satiation (Allan and Rowland, 1992). Water quality parameters such as water temperature, pH and dissolved oxygen were monitored using pH meter (Jenway 3510), DO meter (Jenway 970) and electrical conductivity (EC) meter (HANNA: HI 8633) on daily basis. Aeration (24 h) was provided to all the experimental tanks through capillary system. The range of water quality parameters (pH 7.4-8.6), dissolved oxygen (5.8 - 7.3 mg L⁻¹), electrical conductivity (1.30-1.52 dS m⁻¹) and temperature (24.9-28.7ºC) was noted on daily basis.

Experimental design
Cottonseed meal (CM) was used as test ingredient to formulate experimental diet. Feed was divided into three groups, each having 3 kg weights. 0g (0%), 60g (2%) and 120g (4%) citric acid was added respectively. Experimental diet was divided into nine test diets by supplementing with citric acid (0%, 2% and 4%) and graded (0, 400 and 800 FTU kg⁻¹) levels of phytase. Nine cottonseed meal based citric acid and phytase supplemented test diets were fed to nine groups of fish stocked in experimental tanks. Each of the treatment and control diet had three replicates with 15 fingerlings in each replicate. Total duration of the experiment was ten weeks. Cottonseed meal based citric acid and phytase supplemented diets were compared with each other to determine mineral digestibility parameters using Completely Randomized Design (CRD).

Feed ingredients and formulation of experimental diets
The feed ingredients were purchased from a commercial feed mill and were analyzed for chemical composition following AOAC (1995) prior to the formulation of the experimental diets (Table 1). The feed ingredients were finely grinded to pass through
0.5 mm sieve size. Feed was divided into three groups, each having 3 kg weights. 0g (0%), 60g (2%) and 120g (4%) citric acid was added respectively. All ingredients were mixed in an electric mixer for 10 min and fish oil was gradually added during mixing of the diet. During mixing of ingredients, 10-15% water was also added and the mixture was then extruded using SYSLG30-IV experimental extruder to produce floating pellets (3 mm). The above procedure was followed to formulate the nine cottonseed meal based test diets. The required concentrations (0, 400 and 800 FTU kg^{-1}) of phytase (Phyzyme® XP 10000 FTU g^{-1}; Danisco Animal Nutrition, Fin-65101 Vaasa, Finland) 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 a similar amount of distilled water to maintain an equal level of moisture contents. All the prepared diets were dried and stored at 4°C until use.

**Chemical analysis of feed and feces**

The samples of fish feed ingredients, experimental diets and feces were homogenized using mortar and pestle and analyzed following standard methods (AOAC, 1995). 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 such as calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) were estimated using Atomic Absorption Spectrophotometer (Hitachi Polarized Atomic Absorption Spectrometer, Z-8200). Calibrated standards for mineral estimation were prepared from commercially available standards (AppliChem® Gmbh Ottoweg4, DE-64291 Darmstadt, Germany). The estimation of Na and K was done through flame photometer (Jenway PFP-7, UK). Phosphorus (P) was analyzed calorimetrically (UV/VIS spectrophotometer) using ammonium molybdate as reagent at 720 nm absorbance through standard methods (AOAC, 1995). Chromic oxide contents in diets and feces were estimated after oxidation with molybdate reagent using a UV-VIS 2001 Spectrophotometer at 370nm absorbance (Divakaran et al., 2002).

**Calculation of apparent digestibility coefficient (ADC)**

Apparent minerals digestibility coefficients (ADC) of test diets were calculated by the standard formula (NRC, 1993).

\[
ADC(%) = \frac{100 - 100 \times \text{Percent marker in diet} \times \text{Percent nutrient in feces}}{\text{Percent marker in feces} - \text{Percent nutrient in diet}}
\]

**Statistical analysis**

Resulting data were subjected to two-way Analysis of Variance (ANOVA) (Steel et al., 1996). The differences among means were compared by Tukey’s Honesty Significant Difference Test and considered significant at \( p<0.05 \) (Snedecor and Cochran, 1991). The CoStat computer software (Version 6.303, PMB 320, Monterey, CA, 93940 USA) was used for statistical analysis.

**Results**

Analyzed mineral composition (%) of cottonseed meal based test diets and feces are presented in Table 3 and Table 4 respectively. An interaction between citric acid and phytase was observed for mineral digestibility. It was observed that phytase supplementation reduced the amount of minerals in fish feces at low pH of digestive system.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal</td>
<td>12</td>
</tr>
<tr>
<td>Cottonseed meal</td>
<td>56</td>
</tr>
<tr>
<td>Rice polish</td>
<td>12</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>10</td>
</tr>
<tr>
<td>Fish oil</td>
<td>6</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>1</td>
</tr>
<tr>
<td>Minerals</td>
<td>1</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>1</td>
</tr>
<tr>
<td>Chromic oxide</td>
<td>1</td>
</tr>
</tbody>
</table>
However, further higher levels of phytase and citric acid supplementation were unable to cause any further decrease in mineral contents in feces. Reduced mineral excretion through fish feces was probably due to the hydrolysis of phytate contents by the phytase enzyme supplementation at low pH of digestive system and so more minerals were utilized by *L. rohita* fingerlings. Mineral digestibility (%) of cottonseed meal based test diets in response to phytase and citric acid supplementation is presented in Table 5. Maximum digestibility values (%) of Ca, P, Na, K, Mg, Cu, Mn, Zn and Fe among treatments were observed at 400 FTU kg$^{-1}$ phytase and 4 % of citric acid level. Findings of the present research concluded that phytase and CA at the level of 400 FTU Kg$^{-1}$ and 4% respectively are optimum levels for mineral digestibility.

### Table 2. Chemical analysis (%) of feed ingredients.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Dry matter (%)</th>
<th>Crude Protein (%)</th>
<th>Crude Fat (%)</th>
<th>Crude Fiber (%)</th>
<th>Ash (%)</th>
<th>Gross Energy (kcal/g)</th>
<th>Carbohydrates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal</td>
<td>991.63</td>
<td>448.15</td>
<td>77.16</td>
<td>11.07</td>
<td>26.73</td>
<td>2.69</td>
<td>16.89</td>
</tr>
<tr>
<td>Rice polish</td>
<td>994.09</td>
<td>112.35</td>
<td>113.54</td>
<td>112.70</td>
<td>10.18</td>
<td>3.33</td>
<td>51.23</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>992.45</td>
<td>110.10</td>
<td>22.35</td>
<td>22.65</td>
<td>2.08</td>
<td>2.96</td>
<td>82.82</td>
</tr>
<tr>
<td>Cottonseed meal</td>
<td>993.71</td>
<td>229.59</td>
<td>25.46</td>
<td>16.53</td>
<td>14.56</td>
<td>4.87</td>
<td>43.86</td>
</tr>
</tbody>
</table>

### Table 3. Analyzed minerals composition (%) in test diets of *Labeo rohita* fingerlings fed on cottonseed meal based test diets supplemented with graded levels of citric acid and phytase.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Citric acid</th>
<th>Pytase FTU Kg$^{-1}$</th>
<th>Ca (%)</th>
<th>P (%)</th>
<th>Na (%)</th>
<th>K (%)</th>
<th>Mg (%)</th>
<th>Fe (%)</th>
<th>Cu (%)</th>
<th>Mn (%)</th>
<th>Zn (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>0%</td>
<td></td>
<td>0.132</td>
<td>1.886</td>
<td>0.798</td>
<td>1.160</td>
<td>0.068</td>
<td>0.063</td>
<td>0.142</td>
<td>0.065</td>
<td>0.062</td>
</tr>
<tr>
<td>D2</td>
<td>400</td>
<td>0.133</td>
<td>1.889</td>
<td>0.793</td>
<td>1.164</td>
<td>0.075</td>
<td>0.062</td>
<td>0.142</td>
<td>0.070</td>
<td>0.062</td>
<td></td>
</tr>
<tr>
<td>D3</td>
<td>800</td>
<td>0.133</td>
<td>1.875</td>
<td>0.788</td>
<td>1.168</td>
<td>0.067</td>
<td>0.062</td>
<td>0.142</td>
<td>0.074</td>
<td>0.060</td>
<td></td>
</tr>
<tr>
<td>D4</td>
<td>0%</td>
<td></td>
<td>0.133</td>
<td>1.876</td>
<td>0.795</td>
<td>1.171</td>
<td>0.069</td>
<td>0.062</td>
<td>0.143</td>
<td>0.076</td>
<td>0.063</td>
</tr>
<tr>
<td>D5</td>
<td>400</td>
<td>0.132</td>
<td>1.879</td>
<td>0.773</td>
<td>1.178</td>
<td>0.069</td>
<td>0.064</td>
<td>0.142</td>
<td>0.080</td>
<td>0.061</td>
<td></td>
</tr>
<tr>
<td>D6</td>
<td>800</td>
<td>0.133</td>
<td>1.880</td>
<td>0.784</td>
<td>1.178</td>
<td>0.068</td>
<td>0.064</td>
<td>0.143</td>
<td>0.081</td>
<td>0.063</td>
<td></td>
</tr>
<tr>
<td>D7</td>
<td>0%</td>
<td></td>
<td>0.133</td>
<td>1.882</td>
<td>0.798</td>
<td>1.182</td>
<td>0.068</td>
<td>0.061</td>
<td>0.143</td>
<td>0.083</td>
<td>0.062</td>
</tr>
<tr>
<td>D8</td>
<td>400</td>
<td>0.132</td>
<td>1.872</td>
<td>0.776</td>
<td>1.166</td>
<td>0.071</td>
<td>0.062</td>
<td>0.142</td>
<td>0.084</td>
<td>0.065</td>
<td></td>
</tr>
<tr>
<td>D9</td>
<td>800</td>
<td>0.133</td>
<td>1.873</td>
<td>0.771</td>
<td>1.170</td>
<td>0.070</td>
<td>0.057</td>
<td>0.143</td>
<td>0.084</td>
<td>0.062</td>
<td></td>
</tr>
</tbody>
</table>

LSD value for 0.05=0.19

Data are means of three replicates.

### Discussion

In the present study, improved mineral digestibility was observed in phytase treated groups with supplementation of citric acid as compared to control diet. It is well known that phytate chelates with minerals and makes them unavailable to fish by reducing their digestibility (Cao et al., 2007; Hussain et al., 2011b). In the present study, phytase inclusion at the level of 400 FTUkg$^{-1}$ and 4% citric acid efficiently increased mineral contents in *Labeo rohita* fingerlings. Addition of citric acid to the diet reduces the pH of stomach and enhances the phytase activity to break the phytate complexes (Baruah et al., 2005). In addition to this, the epithelial cell multiplying in the GIT mucosa is also stimulated by citric acid and thus the digestibility of minerals enhances (Sakata et al., 1995). The results of present research proved the combine effect of citric acid and phytase because the digestibility 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 and in case of cottonseed meal based diet highest mineral digestibility values were at 4% citric acid and 400 FTU kg$^{-1}$ level. Debnath et al.
(2007) and Sardar et al. (2006) found that supplementation of dietary microbial phytase at 500 FTU kg\(^{-1}\) level improved the absorption of minerals such as Fe and other minerals such as Na, K, P, Mg, Mn. Gao et al. (2006) found that phytase supplementation between 500 and 1000 FTU kg\(^{-1}\) levels had significantly increased phosphorus availability to fish and reduced the phosphorus excretion through feces. Baruah et al. (2007) found maximum digestibility of major minerals at 3% citric acid and 500 FTU kg\(^{-1}\) phytase level.

Table 4. Analyzed mineral composition (%) in the feces of Labeo rohita fingerlings fed on cottonseed meal based test diets supplemented with graded levels of citric acid and phytase.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Citric acid</th>
<th>Pytase FTU Kg(^{-1})</th>
<th>Ca</th>
<th>P</th>
<th>Na</th>
<th>K</th>
<th>Mg</th>
<th>Fe</th>
<th>Cu</th>
<th>Mn</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>0</td>
<td>0.086</td>
<td>1.23</td>
<td>0.415</td>
<td>0.723</td>
<td>0.052</td>
<td>0.048</td>
<td>0.096</td>
<td>0.049</td>
<td>0.045</td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>0%</td>
<td>400</td>
<td>0.053</td>
<td>0.715</td>
<td>0.291</td>
<td>0.578</td>
<td>0.042</td>
<td>0.087</td>
<td>0.028</td>
<td>0.029</td>
<td></td>
</tr>
<tr>
<td>D3</td>
<td>2%</td>
<td>800</td>
<td>0.062</td>
<td>0.815</td>
<td>0.347</td>
<td>0.656</td>
<td>0.047</td>
<td>0.031</td>
<td>0.072</td>
<td>0.037</td>
<td>0.033</td>
</tr>
<tr>
<td>D4</td>
<td>4%</td>
<td>0.075</td>
<td>0.950</td>
<td>0.362</td>
<td>0.688</td>
<td>0.051</td>
<td>0.039</td>
<td>0.085</td>
<td>0.042</td>
<td>0.039</td>
<td></td>
</tr>
<tr>
<td>D5</td>
<td>6%</td>
<td>0.045</td>
<td>0.564</td>
<td>0.340</td>
<td>0.484</td>
<td>0.035</td>
<td>0.021</td>
<td>0.068</td>
<td>0.026</td>
<td>0.023</td>
<td></td>
</tr>
<tr>
<td>D6</td>
<td>8%</td>
<td>0.051</td>
<td>0.657</td>
<td>0.272</td>
<td>0.546</td>
<td>0.038</td>
<td>0.024</td>
<td>0.061</td>
<td>0.032</td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td>D7</td>
<td>10%</td>
<td>0.059</td>
<td>0.789</td>
<td>0.287</td>
<td>0.644</td>
<td>0.046</td>
<td>0.029</td>
<td>0.081</td>
<td>0.039</td>
<td>0.033</td>
<td></td>
</tr>
<tr>
<td>D8</td>
<td>12%</td>
<td>0.033</td>
<td>0.498</td>
<td>0.202</td>
<td>0.427</td>
<td>0.031</td>
<td>0.018</td>
<td>0.050</td>
<td>0.024</td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>D9</td>
<td>14%</td>
<td>0.048</td>
<td>0.613</td>
<td>0.257</td>
<td>0.526</td>
<td>0.038</td>
<td>0.021</td>
<td>0.035</td>
<td>0.031</td>
<td>0.025</td>
<td></td>
</tr>
</tbody>
</table>

Saba (2011) found maximum digestibility of minerals at 3% citric acid and 750 FTU kg\(^{-1}\) level in sunflower meal fed to Labeo rohita fingerlings. Rashid (2012) reported maximum digestibility values of minerals at 5% citric acid and 750 FTU kg\(^{-1}\) phytase level. Shah et al. (2015) concluded that citric acid and phytase supplemented soybean meal based diet showed the potential to improve muscle mineralization of Labeo rohita juveniles individually as well as synergistically. So the results of current work are supported by above studies and minute variations might be associated with difference in diet composition, fish species and rearing conditions. According to Sugiuara et al. (2001) citric acid and phytase synergistically improved the digestibility of minerals (except Cu and Zn) in fish. Baruah et al. (2007) reported a significant synergistic effects of citric acid (3%) and phytase (500 FTU kg\(^{-1}\)) on absorption of P, Na, K, Mg, Mn, and Fe in Labeo rohita fingerlings fed on plant based diet.

Table 5. Mineral digestibility (%) of fish fed on cottonseed meal based test diets supplemented with graded levels of citric acid and phytase.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Citric acid</th>
<th>Pytase FTU Kg(^{-1})</th>
<th>Ca</th>
<th>P</th>
<th>Na</th>
<th>K</th>
<th>Mg</th>
<th>Fe</th>
<th>Cu</th>
<th>Mn</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>0</td>
<td>40.93</td>
<td>64.95</td>
<td>53.04</td>
<td>43.66</td>
<td>30.59</td>
<td>31.33</td>
<td>38.73</td>
<td>32.23</td>
<td>34.40</td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>0%</td>
<td>63.13</td>
<td>60.61</td>
<td>65.60</td>
<td>53.96</td>
<td>47.50</td>
<td>62.63</td>
<td>43.35</td>
<td>62.65</td>
<td>56.42</td>
<td></td>
</tr>
<tr>
<td>D3</td>
<td>2%</td>
<td>800</td>
<td>57.57</td>
<td>60.61</td>
<td>60.12</td>
<td>49.14</td>
<td>36.78</td>
<td>54.45</td>
<td>54.19</td>
<td>55.33</td>
<td>50.35</td>
</tr>
<tr>
<td>D4</td>
<td>4%</td>
<td>0.089</td>
<td>54.23</td>
<td>58.83</td>
<td>46.90</td>
<td>33.20</td>
<td>43.23</td>
<td>46.13</td>
<td>49.81</td>
<td>43.57</td>
<td></td>
</tr>
<tr>
<td>D5</td>
<td>6%</td>
<td>400</td>
<td>68.92</td>
<td>72.44</td>
<td>59.59</td>
<td>62.27</td>
<td>53.20</td>
<td>69.85</td>
<td>56.03</td>
<td>69.79</td>
<td>66.05</td>
</tr>
<tr>
<td>D6</td>
<td>8%</td>
<td>800</td>
<td>68.12</td>
<td>76.68</td>
<td>70.86</td>
<td>61.11</td>
<td>52.40</td>
<td>69.04</td>
<td>64.49</td>
<td>66.83</td>
<td>63.21</td>
</tr>
<tr>
<td>D7</td>
<td>10%</td>
<td>0.093</td>
<td>62.01</td>
<td>67.43</td>
<td>50.59</td>
<td>38.99</td>
<td>56.91</td>
<td>48.76</td>
<td>56.88</td>
<td>52.07</td>
<td></td>
</tr>
<tr>
<td>D8</td>
<td>12%</td>
<td>400</td>
<td>76.60</td>
<td>75.45</td>
<td>76.03</td>
<td>66.25</td>
<td>59.57</td>
<td>73.07</td>
<td>67.45</td>
<td>73.77</td>
<td>69.61</td>
</tr>
<tr>
<td>D9</td>
<td>14%</td>
<td>800</td>
<td>65.92</td>
<td>70.27</td>
<td>60.67</td>
<td>59.17</td>
<td>49.92</td>
<td>66.17</td>
<td>66.31</td>
<td>65.98</td>
<td>63.35</td>
</tr>
</tbody>
</table>

In conclusion, the current study provided sufficient evidences that supplementation of citric acid at 4% and 400 FTU kg\(^{-1}\) level of phytase had a significant effect on the mineral digestibility by Labeo rohita fingerlings fed on cottonseed meal-based diets. It also proves a great relation between citric acid and phytase regarding to increased mineral digestibility in Labeo rohita fed cottonseed meal based diets. Citric
acid and phytase supplementation in plant based diets may decrease the need for supplementation of minerals which will reduce the cost of fish feed and minerals discharge through feces into the aquatic ecosystem resulting in environment friendly aquaculture.

References


http://dx.doi.org/10.1111/j.1365-2109.2005.01290.x


http://dx.doi.org/10.1016/j.enzmicrotec.2007.01.007


http://dx.doi.org/10.1046/j.1365-2095.2002.00219.x


http://dx.doi.org/10.1111/j.1365-2109.2004.01203.x


http://dx.doi.org/10.1111/j.1365-2109.2007.01704.x


Khajepour F, Hosseini SA. 2012b. Calcium and phosphorus status in juvenile Beluga (Huso huso) fed citric acid supplemented diets. Aquaculture Research


Robinson EH, Li MH, Manning BB. 2002. Comparison of microbial phytase and dicalcium phosphate or growth and bone mineralization of

http://dx.doi.org/10.1046/j.1365-2095.2001.00172.x


http://dx.doi.org/10.1016/j.anifeedsci.2013.02.004


Robinson EH, Li MH, Manning BB. 2002. Comparison of microbial phytase and dicalcium phosphate or growth and bone mineralization of


