INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 8, No. 2, p. 25-35, 2016

RESEARCH PAPER OPEN ACCESS

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 Arsalan, 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.

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; Dedeke *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 (Enterria *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 (24.9-28.7^oC) 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).

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

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 1. Composition (%) of cottonseed meal based diets supplemented with phytase and citric acid.

Ingredients	Composition (%)
Fish meal	12
Cottonseed meal	56
Rice polish	12
Wheat flour	10
Fish oil	6
Vitamin premix	1
Minerals	1
Ascorbic acid	1
Chromic oxide	1

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.

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

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.

Diets	Citric acid	Pytase FTU Kg^{-1}	Ca	P	Na	K	Mg	Fe	Cu	Mn	Zn
D1	0%	0	0.132	1.886	0.798	1.160	0.068	0.063	0.142	0.065	0.062
D2		400	0.133	1.889	0.793	1.164	0.075	0.062	0.142	0.070	0.062
D3		800	0.133	1.875	0.788	1.168	0.067	0.062	0.142	0.074	0.060
D4	2%	0	0.133	1.876	0.795	1.171	0.069	0.062	0.143	0.076	0.063
D5		400	0.132	1.879	0.773	1.178	0.069	0.064	0.142	0.080	0.061
D6		800	0.133	1.880	0.784	1.178	0.068	0.064	0.143	0.081	0.063
D7	4%	0	0.133	1.882	0.798	1.182	0.068	0.061	0.143	0.083	0.062
D8		400	0.132	1.872	0.776	1.166	0.071	0.062	0.142	0.084	0.065
D9		800	0.133	1.873	0.771	1.170	0.070	0.057	0.143	0.084	0.062

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.*

(2005), Ai *et al.* (2007) and Sardar *et al.* (2007) found that supplementation of dietary microbial phytase at 500 FTUkg^{-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 phosphorous availability to fish and reduced the phosphorous 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.

Diets	Citric acid	Pytase FTU Kg^{-1}	Ca	P	Na	K	Mg	Fe	Cu	Mn	Zn
D1	0%	0	0.086	1.233	0.415	0.723	0.052	0.048	0.096	0.049	0.045
D2		400	0.053	0.715	0.291	0.578	0.042	0.025	0.087	0.028	0.029
D3		800	0.062	0.815	0.347	0.656	0.047	0.031	0.072	0.037	0.033
D4	2%	0	0.075	0.950	0.362	0.688	0.051	0.039	0.085	0.042	0.039
D5		400	0.045	0.564	0.340	0.484	0.035	0.021	0.068	0.026	0.023
D6		800	0.051	0.657	0.272	0.546	0.038	0.024	0.061	0.032	0.028
D7	4%	0	0.059	0.789	0.287	0.644	0.046	0.029	0.081	0.039	0.033
D8		400	0.033	0.498	0.202	0.427	0.031	0.018	0.050	0.024	0.021
D9		800	0.048	0.613	0.257	0.526	0.038	0.021	0.053	0.031	0.025

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 Sugiura *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.

Diets	Citric acid	Pytase FTU Kg^{-1}	Ca	P	Na	K	Mg	Fe	Cu	Mn	Zn
D1	0%	0	40.93	40.95	53.04	43.66	30.59	31.33	38.73	32.23	34.40
D2		400	63.13	64.91	65.96	53.96	47.50	62.63	43.35	62.65	56.42
D3		800	57.57	60.61	60.12	49.14	36.78	54.45	54.19	55.33	50.35
D4	2%	0	48.89	54.23	58.83	46.90	33.20	43.23	46.13	49.81	43.67
D5		400	68.92	72.44	59.59	62.27	53.20	69.85	56.03	69.79	66.05
D6		800	68.12	70.68	70.86	61.11	52.40	69.04	64.49	66.83	63.21
D7	4%	0	59.93	62.01	67.43	50.59	38.99	56.91	48.76	56.88	52.07
D8		400	76.60	75.45	76.03	66.25	59.57	73.07	67.45	73.77	69.61
D9		800	65.92	70.27	69.67	59.17	49.92	66.17	66.31	65.98	63.35

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

Abid M, Ahmed MS. 2009. Growth response of *Labeo rohita* fingerlings fed with different feeding regimes under intensive rearing. Journal of Animal and Plant Science **19,** 45-49.

Ai Q, Mai K, Zhang W, Xu W, Tan B, Zhang C, Li H. 2007. Effects of exogenous enzymes (phytase, non-starch polysaccharide enzyme) in diets on growth, feed utilization, nitrogen and phosphorus excretion of Japanese seabass, *Lateolabrax japonicus*. Comparative Biochemistry and Physiology Part A **147,** 502-508.
http://dx.doi.org/10.1016/j.cbpa.2007.01.026

Allan GL, Rowland SJ. 1992. Development of an experimental diet for silver perch *(Bidynus bidyanus)*. Austasia Aquaculture **6,** 39-40.

Association of Official Analytical Chemists, AOAC. 1995. Official Methods of Analysis. 15th Ed. Washington, D.C. USA. 1094 .

Barnes ME, Brown ML, Rosentrater KA. 2012. Juveniles of rainbow trout responses to diets containing distillers dried grain with soluble, phytase and amino acid supplements. Open Journal of Animal Science **2,** 69-77.
http://dx.doi.org/10.4236/ojas.2012.22011

Baruah K, Pal AK, Sahu NP, Debnath D, Yengkokpam S. 2007. Interactions of dietary microbial phytase, citric acid and crude protein level on mineral utilization by Rohu, *Labeo rohita* (Hamilton), juveniles. Journal of World Aquaculture Society **38,** 238–249.
http://dx.doi.org/10.1111/j.1749-7345.2006.00081.x

Baruah K, Pal AK, Sahu NP, Jain KK, Mukherjee SC, Debnath D. 2005. Dietary protein level, microbial phytase, citric acid and their interactions on bone mineralization of *Labeo rohita* (Hamilton) juveniles. Aquaculture Research **36,** 803–812.
http://dx.doi.org/10.1111/j.1365-2109.2005.01290.x

Baruah K, Sahu NP, Pal AK, Debnath D. 2004. Dietary phytase: An ideal approach for cost effective and low-polluting aqua-feed. NAGA, World Fish Center Quarterly **27,** 15-19.

Caipang CMA, Dechavez RB, Amar MJA. 2011. Potential application of microbial phytase in aquaculture. International Journal of Bioflux Society **3,** 55-66.

Cao L, Wang W, Yang C, Yang Y, Diana J, Yakupitiyage A, Luo Z, Li D, 2007. Application of microbial phytase in fish feed. Enzyme Microbial Technology **14,** 342-362.
http://dx.doi.org/10.1016/j.enzmictec.2007.01.007

Cheng ZJ, Hardy RW. 2002. Effect of microbial phytase on apparent nutrient digestibility of barley, canola meal, wheat and wheat middlings, measured in vivo using rainbow trout (*Oncorhynchus mykiss*). Aquaculture Nutrition **8,** 271-277.
http://dx.doi.org/10.1046/j.1365-2095.2002.00219.x

Debnath D, Pal AK, Sahu NP, Jain KK, Yengkokpam S, Mukherjee SC. 2005. Effects of dietry microbial phytase supplementation on growth and nutrient digestibility of *Pangasius pangasius* (Hamilton) fingerlings. Aquaculture Research **36,** 180-187.
http://dx.doi.org/10.1111/j.1365-2109.2004.01203.x

Dedeke GA, Owa SO, Olurin KB, Akinfe AO, Awotedu OO. 2013. Partial replacement of fish meal by earthworm meal (*Libyodrilus violaceus*) in diets for African catfish (*Clarias gariepinus)*. International Journal of Fisheries and Aquaculture **5,** 229-233.

Divakaran S, Leonard GO, Ian PF. 2002. Note on the methods for determination of chromic oxide in shrimp feeds. Journal of Agricultural and Food Chemistry **50,** 464-467.
http://dx.doi.org/10.1021/jf011112s

El-Saidy, DMSD, Gaber MMA. 2004. Use of cottonseed meal supplement with iron for detoxification of gossypol as a replacement of fish meal in Nile tilapia, *Oreochromis niloticus* (L.) diets. Aquaculture Research **35,** 859-869.
http://dx.doi.org/10.1111/j.1365-2109.2004.01077.x

Enterria A, Slocumm, David A, Panayotis B, Karayannakidis D, Lee CM. 2011. Partial Replacement of Fish Meal with Plant Protein Sources Singly and in Combination in Diets for Summer Flounder (*Paralichthys dentatus*). World Aquaculture Society **42,** 753-765.
http://dx.doi.org/10.1111/j.1749-7345.2011.00533.x

Erdman JW, Jr. 1979. Oilseed phytates: nutritional implications. Journal of the American Oil Chemists' Society **56,** 736–741.
http://dx.doi.org/10.1007/bf02663052

Essa AM, Mabrouk AH, Zaki AM. 2004. Growth performance of grass carp (*Ctenopharyngodon idella)* and hybrid grass carp fingerlings fed on different types of aquatic plants and artificial diet in concrete basins. Egyptian Journal of Aquatic Research **30,** 341-348.

Gao C, Chuan-bin X, Yan-ling W, Guang-li F, Jian-hua L, Zhong-hu L. 2006. Application of phytase in diets of grass carp. Hubei Agricultural Science **24,** 30–39.

Gatlin III DM, Barrows FT, Brown P, Dabrowski K, Gaylord TG, Hardy RW, Herman E, Hu G, Krogdahl A, Nelson R, Overturf K, Rust M, Sealey W, Skonberg D, Souza EJ, Stone D, Wilson R, Wurtele E. 2007. Expanding the utilization of sustainable plant products in aqua feeds: a review. Aquaculture Research **38,** 551–579.
http://dx.doi.org/10.1111/j.1365-2109.2007.01704.x

Hussain SM, Afzal M, Rana SA, Javid A, Iqbal M. 2011b. Effect of phytase supplementation on growth performance and nutrient digestibility of *Labeo rohita* fingerlings fed on corn gluten meal-based diets. International Journal of Agriculture Biology **13,** 916–922.

Hussain SM, Javed M, Javid A, Javid T, Hussain N. 2011a. Growth responses of *Catla catla*, *Labeo rohita* and *Cirrhinas mrigala* during chronic exposure of iron. Pakistan Journal of Agriculture Sciences **48,** 239–244.

Hussain SM, Shahzad MM, Afzal M, Javid A, Mubarik MS, Shah SZH, Hussain M, Ahmad S, Arsalan MZH, Manzoor R, Riaz D. (2015). Efficacy of Phytase Enzyme for Increasing Mineral Digestibility of *Cirrhinus mrigala* Fingerlings Fed on Soybean Meal-Based Diet. Pakistan Journal of Zoology **47,** 1807-1816.

Hussain SM, Shahzad, MM, Jabeen F, Nasir S, Afzal M, Javid A, Ahmad S, Arsalan MZH, Riaz D, Khichi TAA, Ahmad AW, Furqan M. (2014). Growth performance and nutrient digestibility of *Cirrhinus mrigala* fingerlings fed on soybean meal-based diet supplemented by phytase. International Journal of Biosciences **12,** 212-221.
http://dx.doi.org/10.12692/ijb/5.12.212-221

Iqbal KJ, Ashraf M, Abbas F, Javid A, Hafeez-Ur-Rehman M, Abbas S, Rasool F, Khan N, Khan SA, Altaf M. 2014. Effect of plant-fishmeal and plant by-product based feed on growth, body composition and organoleptic flesh qualities of *Labeo rohita*. Pakistan Journal of Zoology **46,** 253-260.

Ji H. 1999. Anti-nutritional factors in plant based fish feed. Fish Reserve **19,** 22–24.

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

43, 407–411.
http://dx.doi.org/10.1111/j.1365-2109.2011.02843.x

Khajepour F, Hosseini SA. Imanpour MR. 2012a. Dietary crude protein, citric acid and microbial phytase and their interacts to influence growth performance, muscle proximate composition and hematocrit of common carp, *Cyprinus carpio* L, juveniles. World Journal of Zoology **7,** 118-122.

Lech PL, Reigh RC. 2012. Plant products affect growth and digestive efficiency of cultured Florida pompano (*Trachinotus carolinus*) fed compounded diets. PLoS One, **7,** e34981.
http://dx.doi.org/10.1371/journal.pone.0034981

Lee KJ, Rinchard J, Dabrowski K, Babiak I, Ottobre JS, Christensen JE. 2006. Long-term effects of dietary cottonseed meal on growth and reproductive performance of rainbow trout. Animal Feed Science and Technology **126,** 93-106.
http://dx.doi.org/10.1016/j.anifeedsci.2005.06.007

Li MH, Robinson EH. 2006. Use of Cottonseed Meal in Aquatic Animal Diets: a Review. North American Journal of Aquaculture **68,** 14–22. http://dx.doi.org/10.1577/A05-028.1

Lim SJ, Lee KJ. 2008. Supplemental iron and phosphorus increase dietary inclusion of cottonseed and soybean meal in olive flounder (*Paralichthys olivaceus*). Aquaculture Nutrition **14,** 423–430.
http://dx.doi.org/10.1111/j.1365-2095.2007.00546.x

Lim SJ, Lee KJ. 2009. Partial replacement of fish meal by cottonseed meal and soybean meal with iron and phytase supplementation for parrot fish *Oplegnathus fasciatus*. Aquaculture **290,** 283-289.
http://dx.doi.org/10.1016/j.aquaculture.2009.02018

Mbahinzireki GB, Dabrowski K, Lee KJ, El-Saidy D, Wisner ER. 2001. Growth, feed utilization and body composition of Tilapia (*Oreochromis* sp.) fed with cottonseed meal-based diets in a reticulating system. Aquaculture Nutrition **7,** 189–200.
http://dx.doi.org/10.1046/j.1365-2095.2001.00172.x

Morales GA, Saenz M, Márquez L, Díaz M Moyano FJ. 2013. Solubilisation of protein fractions induced by *Escherichia coli* phytase and its effects on in vitro fish digestion of plant proteins. Journal of Animal Feed Science **10,** 10-16.
http://dx.doi.org/10.1016/j.anifeedsci.2013.02.004

National Research Council, NRC. 1993. Nutrient Requirements of Fish, 114. Washington, DC, National Academy Press.

Nwanna LC, Fagbenro OA, Adeyo AO. 2005. Effects of different treatments of dietary soybean meal and phytase on the growth and mineral deposition in African Catfish (*Clarias gariepinus*). Journal of Animal and Veterinary Advances **4,** 980-987.

Pham MA, Lee KJ, Dang TM, Lim SJ, Ko GY, EO J, Oh DH. 2008. Improved apparent digestibility coefficient of protein and phosphorus by supplementation of microbial phytase in diets containing cottonseed and soybean meal for juvenile olive flounder (*Paralichthys olivaceus*).Asian-Australas. Journal of Animal Science **21,** 1367–1375.
http://dx.doi.org/10.5713/ajas.2008.80053

Phromkunthong W, Nuntapong N, Gabaudan J. 2010. Interaction of phytase RONOZYME® P (L) and citric acid on the utilization of phosphorus by common carp (*Cyprinus carpio*). Songklanakarin. Journal of Science and Technology **32,** 547–554.

Rawles SD, Gatlin DM. 2000. Nutrient digestibility of common feed stuffs in extruded diets for sunshine bass (*Morone chrysops* × *M. saxatilis*). Journal of World Aquaculture Society **31,** 570–579.
http://dx.doi.org/10.1111/j.17497345.2000.tb00906.x

Robinson EH, Li MH, Manning BB. 2002. Comparison of microbial phytase and dicalcium phosphate or growth and bone mineralization of pond-raised channel catfish, *Ictalurus punctatus*. Journal of Applied Aquaculture **12,** 81–88. http://dx.doi.org/10.1300/j028v12n03_08

Rowland SJ, Ingram BA. 1991. Diseases of Australian native freshwater fishes with particular emphasis on the ecto-parasitic and fungal diseases of Murray cod (*Maccullochella peeli*), golden perch (*Macquaria ambigua*) and silver perch (*Bidyanus bidyanus*).*NSW* Fisheries Bulletin **4,** 1-29.

Saeed MR. 2012. Influence of citric acid supplementation on growth performance, minerals and protein digestibility of phytase treated sunflower meal based diet fed to Labeo rohita fingerlings. *MPhil Thesis*. University of Agriculture, Faisalabad, Pakistan.

Sakata T, Adachi M, Hashida M, Sato N, Kojima T. 1995. Effect of n-butyric acid on epithelial cell proliferationof pig colonic mucosa in short-term culture. Deutsche tierarztliche Wochenschrift **102,** 163-164.

Sardar PH, Randhawa S, Abid M, Prabhakar SK. 2007. Effect of dietary microbial phytase supplementation on growth performance, nutrient utilization, body compositions and haematobiochemical profiles of *Cyprinus carpio* (L.) fingerlings fed soy protein-based diet. Aquaculture Nutrition **13,** 444-456. http://dx.doi.org/10.1111/j.1365-2095.2007.00497.x

Shah SZH, Afzal M, Shafaat YK, Hussain SM, Zeeshan R. 2015. "Prospects of Using Citric Acid as Fish Feed Supplement." International Journal of Agriculture Biology **17,** 1-8.

Shapawi R, Ebi I, Yong A, 2013. Soybean meal as a source of protein in formulated diets for tiger grouper, *Epinephelus fuscoguttatus* juvenile. Part I: Effects on growth, survival, feed utilization and body compositions. Agricultural Sciences **4,** 317-323. http://dx.doi.org/10.4236/as.2013.46a003

Silva LG, Trugo LC, Terzi SC, Couri S. 2005. Low phytate lupin flour based biomass obtained by fermentation with mutant of *Aspergillus niger*. Process Biochemistry **40,** 951-954. http://dx.doi.org/10.1016/j.procbio.2004.02.016

Snedecor GW, Cochran WG. 1991. Statistical Methods. 8th Ed. Iowa State University. Press, Americans.USA, 503.

Steel RGD, Torrie JH, dickey DA. 1996. Principles oand procedures of statistics, 3rd Ed. McGraw Hill international Book Co. Inc., *New York*. USA. 336-352.

Sugiura SH, Gabaudan J, Dong FM, Hardy RW. 2001. Dietary microbial phytase supplementation and the utilization of phosphorus, trace minerals and protein by rainbow trout *Oncorhynchus mykiss* (W) fed soybean meal-based diets. Aquaculture Research **32,** 583-592. http://dx.doi.org/10.1046/j.1365-2109.2001.00581.x

Sugiura SH, Roy PK, Ferraris RP. 2006. Dietary acidification enhances phosphorus digestibility but decreases H+/K+-ATPase expression in rainbow trout. Journal of Experimental Biology **209,** 3719–3728. http://dx.doi.org/10.1242/jeb.02436

Vandenberg WG, Scott SL, Noue JD. 2011. Factors affecting nutrient digestibility in rainbow trout (*Oncorhynchus mykiss*) fed a plant protein-based diet supplemented with microbial phytase. Aquaculture Nutrition **18,** 369-379. http://dx.doi.org/10.1111/j.1365-2095.2011.00901.x

Vielma J, Ruohonen K, Gabaudan J, Vogel K. 2004. Top spraying soybean meal-based diets with phytase improves protein and mineral digestibilities but not lysine utilization in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture Research **35,** 955-964. http://dx.doi.org/10.1111/j.1365-2109.2004.01106.x

Wang Y, Yu S, Wang Y, Che J, Zhao L, Bu X, Yang Y. 2015. Effect of replacing fish meal with soybean meal on growth, feed utilization and nitrogen and phosphorus excretion of juvenile *Pseudobagrus ussuriensis*. Aquaculture Research. 1–11. http://dx.doi.org/doi:10.1111/are.12765

Yildirim O, Acar U, Turker A, Sunar MC, Kesbiç OS. 2014. Effects of replacing fish meal with peanut meal (*Arachis hypogaea*) on growth, feed utilization and body composition of mozambique tilapia fries (*Oreochromis mossambicus*). Pakistan Journal of Zoology **46,** 497-502.

Zhou QC, Tan BP, Mai KS, Liu YJ. 2004. Apparent digestibility of selected feed ingredients for juvenile cobia *(Rachycentron canadum)*. Aquaculture **241,** 441-451. http://dx.doi.org/10.1016/j.aquaculture.2004.08.044