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ENHANCED DIGESTIBILITY OF PHYTASE TREATED CANOLA MEAL BASED DIET FOR *LABEO ROHITA* FINGERLINGS

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ABSTRACT

This study was carried out to enhance the digestibility of nutrients and minerals through acidification of phytase treated canola meal based diet. The canola meal based diet (2% live wet fish weight) was supplemented with two levels of phytase (0 and 500 FTU/kg) and three levels of citric acid (0%, 1.5% and 3%). The optimum level of phytase and citric acid for releasing chelated nutrients and minerals of canola meal based diet were found to be 500 FTU/kg and 3% respectively for *Labeo rohita*. The higher digestibility of nutrients; dry matter, crude protein, crude fat and gross energy and minerals; Ca, Mg, Mn, Fe, Zn, Cu, K, Na and P were absent in groups fed on supplemented diets as compared to the control. These findings provided the strong evidence of significant enhancement of digestibility of nutrients and mineral through acidification of phytase treated diet with citric acid were found to be 500 FTU/kg and 3% citric acid.

Key words: Fish meal; Citric acid; Phytase; Canola diet.

INTRODUCTION

Human population is spreading day by day and people are facing problems of nutrition. The main focus is on protein availability in adequate amount which may negatively affect the anatomy and physiology of human body. To meet the requirement of protein, the most important and easily available source is fish. In order to cope this demand there is need to improve fish production technology through the development of efficient, cost effective and low polluted feed (Lech and Reigh, 2012; Shapawi *et al.*, 2013). Fish meal is a primary animal protein source in aquatic feeds for being richest source of vital nutrients such as crucial amino acid, essential fatty acids, vitamins, minerals and concealed growth issues (Zhou *et al.*, 2004; Drew *et al*., 2007). The present production of fish meal is at maximum level but its use in fish industry is increasing day by day which is reached upto 4 million metric tons by 2015 and total production of fish meal is estimated nearly 6 million tons per year (Ranjhan and Athithan, 2015). Hence due to increased demands, elevated costs and interrupted availability of the fish meal promoted idea to search for another source of protein for fish culture (Pham *et al.*,2008). Moreover, fish meal is expensive than plant protein sources. Plant by-products are outstanding source of protein and energy (Hardy, 2000; Glencross, 2005; Gatlin *et al.*, 2007; Khan *et al*., 2011); Hussain, 2015 a, b), and can be used as alternate source of protein. Canola has comparatively higher protein level than other oilseeds except soybean meal. Drew (2007), unveiled that fish meal is twice the price of canola meal per kg of protein. It has better amino acid profile for animal feeding, but, it has limited amount of lysine. Other than that it has notable level of methionine and cystine. It is highly effective feed ingredient and is obtained by solvent extraction of oil from canola seed (Newkirk, 2009). The portion of seed left after the extraction of oil is meal, effectively used as feed for poultry and livestock. Canola meal accommodates elevated level of fibre such as cellulose (14.5%), hemicellulose (5%), lignin (8.3%), crude fat (3.8%), crude protein (35%), crude fibre (12%) and ash (6%). It constitutes about 15% of total starch, soluble non-starch polysaccharides and free sugars which are helpful to digestible energy. The inclusion of plant proteins in fish feed is prohibited because of the existance of anti-nutritional compounds, such as phytate that may ranges from 5-30 g/kg (Reddy and Sathe, 2002). Canola also contains about 4% phytate or phytic acid which is a common constituent of plant. Phytate is one of the most disturbing factors which destroys the nutritive value of the meal. Phytate is also the main storage form of both phosphorous (P) and inositol in plant seeds and grains, comprising 0.5– 5% (w/w) (Loewus, 2002). This phosphorous is not easily available to stomach less fishes like *Labeo rohita* and other carps. So it remains undigested and expelled out in the form of undigestible phosphorous that imparts in water pollution. The aquatic pollution can be controlled by good uptake of nutrients, hence, the more discharge of P in water can be avoided (Baruah *et al*., 2004). Apart from making poor mineral complexes, phytic acid chelates with protein so that decreased availability of minerals and protein to fish which is considered to be the most vital nutrient (Helland *et al.,* 2006). As a result, the bioavailability of mineral and protein of the feed ingredients to monogastric animals including fish is effectively reduced (Liu *et al.*, 1998; Sugiura *et al,* 2001). It is heat stable anti-nutritional factor and cannot be hydrolysed without enzymatic reactions. Microbial phytase is an enzyme that hydrolyzes phytic acid and release chelated nutrients and mineral in plant protein source. It also has importance to release chelated phosphorous for better utilization of P from plant component (Olusola and Nwanna, 2014; Lim and Lee, 2009). Agastric species of fish is unable to produce this enzyme and therefore cannot utilize better of nutrients and minerals. The supplementation of phytase enzyme in the diet of fish improve the digestibility of nutrients and minerals (Cheng and Hardy, 2003; Yoo *et al.,* 2005; Cao *et al*., 2007) feed conversion efficiency and growth (Sajjadi and Carter, 2004; Schafer *et al.*, 1995; Vielma *et al.*, 1998).

Jongbloed (1987) reported that reducing the intestinal pH through acidification of diet with organic acids, increased the solubility of chelated nutrients, resulted higher absorption of nutrients. The addition of citric acid has been reported to increase the phosphorous absorption by dephosphorylation of phytate (Zyla *et al.*, 1995; Baruah, 2004; Phromkunthong *et al.,* 2010). The optimal level of citric acid reported by (Baruah *et al.*, 2005, 2006) was 3% where phosphorous utilization in *Labeo rohita* juviniles fed soyabean meal based diets was maximum. Positive results were also announced by (Sarker *et al.*, 2005) and (Pandey and Satoh, 2008) on the growth and feed performance by the inclusion of citric acid in fish diets. The feed treated with organic acids has been found useful for better growth, uptake of feed and feed conversion ratio by (Tabrizi *et al.*, 2012).

There is an obvious dearth of reviewed data that accommodates the usage of microbial phytase in combination with citric acid in stomach-less fish like *Labeo rohita,* therefore, the objective of present study was to enhance the digestibility of nutrient of phytase treated canola meal based diet through acidification for *Labeo rohita* fingerlings.

MATERIALS AND METHODS

The present research was carried out to study protein and minerals digestibility of phytase treated canola meal based diet through acidification for *Labeo rohita* fingerlings. The experiments were conducted in the Fish Nutrition Laboratory, Department of Zoology and Fisheries, University of Agriculture, Faisalabad. *Labeo rohita* fingerlings were obtained from Government Fish Seed Hatchery, Faisalabad and acclimatized to experimental conditions in laboratory for two weeks in V-shaped tanks (UA system) specially designed for the collection of fecal material from water media. Twelve fishes were stocked in each tank. During this period the fingerlings were fed once daily to apparent satiation on the basal diet used in subsequent digestibility study (Allan and Rowland, 1992). The fingerlings of *Labeo rohita* were fed at the rate of 2% of live wet weight on their prescribed diets. For each test diet, replicates were assigned. Fecal material of each replicated treatment was collected and dried in oven at 60˚C, ground and stored for chemical analysis. Water quality variables particularly temperature, pH and dissolved oxygen were monitored by the usage of Jenway pH meter model 3510 and D.O. meter model 970, respectively, during the study period. Aeration was provided round-the-clock to all the tanks through capillary system.

Feed Ingredients and Experimental Diets: The feed ingredients were bought from a commercial feed mill and analyzed for chemical composition following (AOAC, 1995) prior to the formulation of the experimental diet. The feed ingredients were ground and sieved to require particle size (0.5mm) before incorporation with experimental diet (Table1).

Table 1. Ingredients composition (%) of canola meal based diet.

Feed Ingredients	Composition(%)
Fish meal	12
Canola meal	56
Rice polish	12
Wheat flour	10
Fish oil	6
Vitamin premix	1
Minerals	1
Ascorbic acid	1
Chromic oxide	1
Total	**100**

All dry ingredients were mixed in electric mixer for 10-20 minutes, where-after fish oil was gradually added, while mixing constantly. Chromic oxide (1%) was incorporated as an inert marker. Citric acid was added at the levels of 0%, 1.5% and 3% to dry mixed ingredients to make three test diets. Ten to fifteen percent water was slowly blended to prepare suitable dough of each test diet; and was further processed through lab extruder for making floating pellets. The pellets of each test diet was sprayed with two phytase levels (0 and 500 FTU/kg diet) resulting the formation of six test diets. The phytase solution was prepared by dissolving 2g of microbial phytase (powder form) into 1000ml of distilled water (Robinson *et al.*, 2002). One unit of phytase activity (FTU) is defined as "the enzyme activity that liberates 1 µmol of inorganic orthophosphates per min at pH 5.5 (37˚C) at a substrate concentration (sodium phosphate) of 5.1 mmol/L." (Engelen *et al.*, 1994).

Analyses of Feed and Feces: For the chemical analyses feed ingredients, test diets and feces were homogenized using pestle and mortar, and analyzed by standard methods (AOAC, 1995). Crude protein by micro Kjeldahl apparatus; crude fat by petroleum ether extraction method (Bligh and Dyer, 1959) 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 with the help of oxygen bomb calorimeter. Moisture was determined by oven-drying at 105˚C for 12 h. Filterate of the samples (Ingredients, experimental diets and feces) was processed for analysis of minerals (AOAC, 1995) and estimated using atomic absorption.

RESULTS

The apparent nutrient digestibility and mineral availability were estimated for the *Labeo rohita* fingerlings fed on Canola meal based test diets supplemented with phytase and citric acid. The percentage of chromic oxide in test diets and feces are shown in Table 2. The chromic oxide (%) ranged from 1.01 to 1.03 in test diets whereas in feces it was 1.12 to 1.33.

Digestibility of Nutrients: The apparent digestibility of all nutrients (%) such as dry matter, crude protein, crude fat and gross energy is given in table 3. The maximum digestibility values of dry matter, crude protein, cude fat and gross energy were achieved for the test diet supplemented with levels of 500 FTU/kg and 3% citric acid. In general, the digestibility pattern was increasing with the increase in concentrations of citric acid and phytase with the lowest digestibility of nutrients at the lowest supplements' levels and high being obtained at highest citric acid and phytase levels.

The maximum dry matter 25.05% was obtained with the highest citric acid concentration (3%) and 500 FTU/kg of phytase when compared to the control value 16.22 % without digestion with citric acid and phytase. Similar trend was obtained for the other parameters studied. Highest crude protein level of 69.98% was achieved with citric acid and phytase treated feed. An increase in the protein levels was corresponding to the increase in the concentration of the citric acid being lowest (50.33%) in absence of citric acid and highest at the higher citric acid levels. Crude fat and gross energy also exhibited the same pattern i.e., both showed the highest values 71.75% and 64.66% respectively at maximum value of citric acid (3%) and 500 FTU/kg of phytase. Lowest digestion of crude fat 55.72% and gross energy 43.54% were obtained when diet is treated without phytase and citric acid. So it is evident from the results that phytase and citric acid played a well role in enhancing the digestion.

The trend when observed for apparent minerals digestibility (such as Ca, Mg, Fe, Mn, Zn, Cu, K, Na and P) by the fish is shown in table 4. Apart from Mg, all other minerals showed highest digestibility at 3% citric acid and 500 FTU/kg of phytase, while magnesium (Mg) showed its maximum digestibility 59.75% at 1.5% citric acid with same phytase level when compared to control diet. The maximum and minimum values obtained for minerals digestibility were 63.00% and 31.81% for Ca, 58.08 % and 20.66% for Fe, 65.25% and 45.04% for Mn, 81.60% and 46.76% for Zn, 52.77% and 36.79% for Cu, 56.95% and 37.99% for K, 60.23% and 30.33% for Na and 76.04% and 3.044% for P, respectively. The variation in digestibility with varied values of citric acid proved that the digestibility of minerals was directly proportional to levels of citric acid which also enhanced the activity of phytase.

Table 2. Percentage of chromic oxide in test diets and feces of *Labeo rohita* fingerlings fed on canola meal based diet.

Citric Acid (%)	Phytase Levels (FTU/kg)	Chromic Oxide (%) in test diet	Chromic Oxide (%) in feces
0	0	1.03±0.010	1.33±0.015
	500	1.03±0.015	1.14±0.025
1.5	0	1.02±0.01	1.13±0.015
	500	1.03±0.005	1.12±0.015
3	0	1.01±0.011	1.36±0.015
	500	1.02±0.005	1.14±0.020

Table 3. Apparent digestibility of nutrients at different citric acid and phytase level.

Citric Acid (%)	Phytase Levels (FTU/kg)	Dry matter (%)	Crude protein (%)	Crude fat (%)	Gross Energy (%)
0	0	16.22±1.68	50.33±1.49	55.72±1.25	43.54±3.34
	500	15.92±2.01	55.28±2.17	59.41±0.12	47.05±3.58
1.5	0	17.27±0.90	54.77±1.43	56.63±1.85	45.60±1.24
	500	19.63±1.88	61.19±0.57	63.46±2.86	53.17±2.92
3	0	21.44±1.80	60.83±2.67	61.29±3.21	56.96±2.95
	500	25.05±1.13	69.98±0.52	71.75±2.14	64.66±1.18

Table 4. Apparent digestibility of minerals at different citric acid and phytase level

Citric acid (%)	Phyt-ase level FTU/kg	Ca Digestibility (%)	Mg Digestibility (%)	Fe Digestibility (%)	Mn Digestibility (%)	Zn Digestibility (%)	Cu Digestibility (%)	K Digestibility (%)	Na Digestibility (%)	P Digestibility (%)
0	0	31.81±2.24	25.71±2.96	20.66±3.92	45.04±2.51	46.76±1.41	36.79±4.42	37.99±1.59	30.33±2.26	34.04±2.12
	500	41.66±3.48	32.41±4.13	21.68±5.32	47.44±2.35	61.19±3.43	38.21±3.06	39.45±1.45	39.29±3.92	37.83±4.06
1.5	0	47.70±1.05	32.41±4.13	28.46±5.28	52.40±3.57	76.92±4.84	36.21±3.06	44.09±1.26	37.57±1.23	42.22±2.60
	500	50.11±5.78	59.75±4.32	37.82±1.68	49.38±4.47	76.21±2.10	48.31±3.32	47.24±2.12	45.85±1.65	56.31±4.46
3	0	42.59±2.70	39.10±5.23	30.61±6.95	46.60±5.53	79.47±2.26	41.31±5.18	48.49±1.98	53.52±3.18	45.39±3.18
	500	63.00±1.98	46.30±4.34	58.08±4.64	65.25±4.62	81.60±3.91	52.77±4.65	56.95±2.05	60.23±2.08	76.04±5.28

DISCUSSION

The whole research was designed to study the effect of phytase and citric acid treated canola meal based diet on the nutrients and mineral digestibility when *Labeo rohita* fingerlings were fed on it. The main reason to choose plant sources in fish feed is that they have less quantity of phosphorous (Wenblad *et al.,* 2013) instead using fishmeal that contains large amount of phosphorous which upon excretion make the water polluted. The higher digestibility of nutrients (crude protein, crude fat and gross energy) and minerals were observed for test diet containing levels of phytase of 500 FTU/kg. The efficiency of phytase was further increased significantly with the addition of organic acid such as citric acid. There are some evidences that the absorption of minerals is affected by the pH of the gastrointestinal tract of fish (Sugiura *et al*., 1998; Vielma *et al*., 1999). Lowering the intestinal pH by the application of citric acid improve absorption of minerals (Jongbloed, 1987). Another effect of citric acids is that it acts as chelating compound (Ravindran and Kornegay, 1993) thus increased the intestinal digestibility of minerals (Sugiura *et al.,* 1998; Vielma *et al.*, 1999), as it lowers intestinal pH and hence increases the phytase performance (Erdman *et al.,* 1979). The results of present study and finding of (Sugiura *et al*., 2001) supported the conclusion that acidification of diet enhanced the performance of microbial phytase.

In present study the higher digestibility of nutrients (dry matter, crude protein, crude fat and gross energy) revealed that the supplementation of phytase (500 FTU/kg) and acidification (3% citric acid) had released chelated nutrients of canola meal based diet and provided the opportunity to the fish to digest the chelated nutrients. The study on crude protein and dry matter was also published by (Khajepour and Hosseini, 2012), which showed the increased digestibility of both when a significant amount of citric acid was added in diet. Formic acid also played an important role in protein digestibility in rainbow trout when observed by (Luckstadt, 2008). Similar findings were also reported by (Farooq, 2012) and (Saeed, 2012) who reported that phytase and citric acid significantly improve the nutrient digestibility in sunflower meal based diet for *labeo rohita at the* optimum levels of 750 FTU/kg and 5% citric acid. . The conclusion can be drawn that the efficiency of phytase might be related to the type of the experimental test diet. Moreover, the activity of phytase seems to be directly proportional to the level of citric acid used. The minerals (Ca, P, Na, K, Fe, Cu, Zn and Mn) digestibility was also highest at 500 FTU/kg of phytase and 3% citric acid except the value (59.75±4.32) of Magnesium (Mg) which was higher at 500 FTU/kg and 1.5% of citric acid. The values revealed that P (76.04±5.28), and Zn (81.60±3.91) in the diet were maximum utilized by *Labeo rohita* fingerlings. It can be concluded from the findings of present study that phytase efficiency for releasing chelated minerals was significantly improved by the acidification with citric acid also favoured by (Phromkunthong *et al.,* 2010), for *Cyprinus carpio.* Similar results were observed by (Baruah *et al*., 2007b) who reported highest bioavailability of major minerals in groups given diets consisting of 500 FTU/kg and 3% citric acid. Saba (2011) also documented higher digestibility of minerals of sunflower meal based diet supplemented with 750 FTU/kg of phytase and 3% citric acid for *Labeo rohita.* Whereas, (Saeed, 2012) also support our study results with his findings of enhanced digestibility of minerals at 750 FTU/kg and 5% citric acid. The combination of phytase and citric acid was experimentally found good for mineral absorption. The levels of phytase used by (Ai *et al.*, 2007) and (Sardar *et al.*, 2007) was 500 FTU/kg and by (Gao *et al*., 2006) were 500 FTU/kg and 1000 FTU/kg which were significantly important in increasing availability of minerals and reducing amount of P in water. The higher digestibility of minerals at different supplementation levels of phytase and citric acid revealed that efficiency of phytase to release maximum chelated minerals from plant based diets depended on the feed ingredient used for the formulation fish feed.

The present research provided the information that the acidification of phytase treated canola meal based diet with citric acid significantly increased the digestibility of nutrient and minerals for *Labeo rohita* in test diet containing 500 FTU/kg and 3% citric acid. The current study showed that 3% citric acid was optimum level for increasing the efficiency of phytase for releasing chelated nutrients and minerals of canola meal based diet.

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