INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 5, No. 12, p. 212-221, 2014

RESEARCH PAPER OPEN ACCESS

Growth performance and nutrient digestibility of *Cirrhinus mrigala* fingerlings fed on soybean meal-based diet supplemented by phytase

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Key words: Phytase, Soybean meal, *Cirrhinus mrigala,* Nutrient digestibility, growth performance.

http://dx.doi.org/10.12692/ijb/5.12.212-221 Article published on December 15, 2014

Abstract

The present study was carried out to evaluate the effects of phytase supplemented soybean meal based diet on growth performance and nutrient digestibility in *Cirrhinus mrigala* fingerlings. Presence of phytic acid in plant ingredients reduces the bioavailability of nutrient to fish as a result reduced fish growth. Reference diet and five test diets were prepared at 30% soybean meal based diet contents to examine the effects of phytase supplementation (0, 500, 1000, 1500 and 2000 FTU kg^{-1}) on plant meal-based diet (soybean meal) and nutrient availability for *Cirrhinus mrigala* fingerlings. Chromic oxide was added as indigestible marker. Triplicate tanks were used for all treatments. Fingerlings were fed at the rate of 5 % of live wet weight of fish. Water quality parameters such as DO, temperature and pH in every tank were monitored using standard methods. The results from the current work showed that 1000 FTU kg^{-1} level in soybean meal based test diets increased the nutrient digestibility in *C. mrigala* as compared to reference diet which resulted in significant ($P<0.05$) increase in growth performance parameters. It is concluded and suggested that the phytase supplemented soybean meal based diet at 1000 FTU kg^{-1} level is optimum to release sufficient chelated nutrients for *C. mrigala* fingerlings.

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Introduction

Indigenous carps of Pakistan like *Cirrhinus mrigala, Labeo rohita* and *Catla catla* are cultivated in governmental and private sector as well as leading the natural water bodies (Khan *et al.,* 2004; Hussain *et al.,* 2011a).

During the last twenty years aquaculture has been well-known as the promoting world food industry (Gatlin *et al.,* 2007; FAO, 2009; Yıldırım *et al*., 2014). We need better quality feed to get maximum yield. Among different feeds fishmeal has been found the major source of protein (Drew *et al.,* 2007). Unfortunately, the fish meal is associated with its instability and high price due to its reduced supply (Shapawi *et al.,* 2013). The main objectives of fisheries industry are to produce high quality fish and to optimize growth (Bello *et al*., 2012). The use of plant meals has been offered as a substitute to fishmeal because they are easily available, have low cost and low phosphorus content as compared to fishmeal (Dalsgaard *et al.,* 2009). Numerous studies have been carried out on nutrient digestibility to investigate the nutritional values of soybean meal for many fish species by replacing fishmeal (Gatlin III *et al.,* 2007; Gaylord and Barrows, 2009; Yue and Zhou, 2009; Antolovic *et al.,* 2012; Banaee *et al.,* 2013; Shapawi *et al.,* 2013). Soybean meal has been recommended as the most favourable, cost effective and good alternative protein source among other plant meals, for fish feed (Hardy, 1996; Khan *et al.,* 2011). Experiments on channel catfish showed that soybean meal that having 28 to 32% protein in crude form principally provides better growth comparable to fish meal (Robinson *et al.,* 2002; Nahashon and Agnes 2013).

Phytate can form phytate-mineral-protein and phytate-protein complexes that create problem in digestion (Laining *et al.,* 2010) as well as phytate may also chelate with amino acids in the different fish species and decreases their availability (Banaee *et al.,* 2013; Shapawi *et al.,* 2013). The main form of stored phosphorus is phytic acid in seeds such as soybean (Jorquera *et al.,* 2008) that usually can cause the decline in apparent protein availability to fish (Gatlin *et al.,* 2007; Laining *et al.,* 2010; Nahashon and Agnes 2013). Mono-gastric fishes poorly consume phosphorus from phytate because they do not have phytases in their digestive tract (Liebert and Portz 2005). This chelated phosphorous is excreted into aquatic environment causing serious threat of pollution and increases the process of eutrophication (Vats *et al.,* 2005). Phytase addition in plant based diets has been resulted to improve the usage of P and bioavailability of nutrients by fish (Cao *et al.,* 2007; Olusola and Nwanna 2014).

Phytase supplementation at 750 FTU kg^{-1} level enhanced the nutrient digestibility of mono-gastric fish that was fed on protein taken from plant sources (Baruah *et al.,* 2007; Hussain *et al.,* 2011a). The body composition and growth performance of *C. mrigala* fingerlings also showed same trends when fed by phytase supplemented diets (Usmani and Jafri, 2002). The ADC (Apparent Digestibility Coefficients) for protein, total phosphorous contents, starch, dry matter, and energy of protein sources were measured by using inert marker like as chromic oxide (Cr_2O_3) (Liu *et al.,* 2013). Research work is needed to uncover the effects of phytase supplemented plant based diets on growth performance, nutrient digestibility and to determine optimum level of phytase for commercially important species like *C. mrigala*.

Materials and methods

Present study was conducted in the Fish Nutrition Laboratory, Department of Zoology, Wildlife and Fisheries, Govt. College University, Faisalabad.

Fish and Acclimatization

Cirrhinus mrigala fingerlings were purchased from Fish Seed Hatchery, Satiana road Faisalabad. The fingerlings were acclimatized in laboratory with experimental conditions for fifteen days in V-shaped fish tanks (GCUF system) that were specially designed for the collection of fecal material. During acclimatization period fish were fed twice daily to apparent satiation level on the basal diet used in subsequent digestibility study (Allan and Rowland,

1992). Water quality parameters particularly water temperature, salt concentration and DO were monitored. Oxygen was provided twenty four hours a day to fish throughout the study period by pump connected with capillary system. NaCl (5 g/L) was used to free *Cirrhinus mrigala* fingerlings from ecto-parasites as well as to prevent fungal infection, before starting experiment (Rowland and Ingram, 1991).

Preparation of Feed

The ingredients of feed were taken from market and tested for chemical composition prior to the formulation of the experimental diets according to AOAC (1995) (Table 1). For normal fish growth reference diet was prepared to supply sufficient level of required nutrients. An inert marker Chromic oxide at the rate of 1% added in reference diet. Test diets were formulated by mixing 70% reference diet and 30% test ingredients (soybean meal) (Table 2) in electric mixture for 8 to 10 minutes along with gradual addition of fish oil. To provide moisture (10-15%) water was also added. The diets were extruded into pellets (3mm) through Lab Extruder (model SYSLG30-IV Experimental Extruder). Five test diets were formulated by spraying different graded levels of phytase at 0, 500, 1000, 1500 and 2000 FTU Kg^{-1}. One unit of phytase activity (FTU) can be defined as the enzyme activity that liberates 1 µmol of inorganic orthophosphate min^{-1} at pH 5.5 (37°C) at a substrate concentration (sodium phosphate) of 5.1 µmol/L (Engelen *et al.,* 1994).

Feeding Protocol

The *Cirrhinus mrigala* fingerlings were fed two times a day (morning & afternoon). In the start of experiment the fish fingerlings were fed at the rate of 5% of live wet weight on their prescribed diet and later adjusted on a daily basis intake of feed by fish. For each test diet every replicate were stocked with fifteen fish (normal weight: 8.02 g/fish). From each tank the uneaten diet was drained out after the feeding period of two and half hours. Before refilling with water, the tanks were washed completely to remove the particles of uneaten diets.

Fecal Collection

Feces were collected from the fecal collection tube of each tank after three hours of feeding time period. Care should be taken avoid breaking of the skinny fecal filaments in order to minimize nutrient leaching. Fecal material of each replicate was dried in oven and stored for further chemical analysis. The experiment was lasted for ten weeks for the collection of 4.5-5.5g feces of each triplicate.

Chemical Analysis of Feed and Feces

By using a motor and pestle the samples of feces, test diets and feed ingredients were homogenized separately and analysed according to AOAC (1995). Crude protein (N x 6.25) was determined by micro kjeldahl apparatus while moisture by oven-drying at 105°C for 12 hours and crude fat was determined by the method of petroleum ether extraction (Bligh and Dyer, 1995) through Soxtec HT2 1045 system. Crude fiber was examined as loss on ignition of dried lipid-free residues after digestion with1.25% H_2SO_4 and 1.25% NaOH; ash by detonation at 650°C temperature for approximately twelve hours in electric furnace (Eyela-TMF 3100). Total carbohydrate (N-free extract) was calculated by difference method i.e.

Total carbohydrate % = 100-(Ether extract %+ Crude protein%+ CF + Ash %).

Oxygen bomb calorimeter was used to determine the gross energy. For calculation of mineral estimation, the diets and feces samples were digested in a boiling nitric acid and per chloric acid mixture (2:1) according to AOAC (1995).

Chromic oxide contents in diets and feces were estimated after oxidation with molybdate reagent (Divakaran *et al.,* 2002) using UV-VIS 2001 Spectrophotometer at 370nm absorbance. Apparent nutrient digestibility coefficients (ADC) of reference and test diets were calculated as follows (NRC, 1993):

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

Statistical Analysis

Finally, data of nutrient digestibility of experimental diets was subjected to one-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 package (Version 6.303, PMB 320, Monterey, CA, 93940 USA) was used for statistical analysis.

Results

The highest weight gain (10.93g) in *C. mrigala* fingerlings were observed in case of soybean meal (30%) based diet containing 1000 FTU kg^{-1} level as compared to other test and reference diets. Though, it was not different significantly from the weight gain observed at 1500 FTU kg^{-1} level. On comparison with other levels, these values showed significant deviations (P<0.05). The weight gain % of the *C. mrigala* found in the various phytase supplemented test diets showed the similar trend as it was observed in case of weight gain. The maximum weight gain % (155%) was observed at 1000 FTU kg^{-1} level test diets, which was higher from all other test diets. The lowest FCR value (1.43) was observed at 1000 FTU kg^{-1} level and it was significantly different (p<0.05) from the FCR values of other test diets as well as reference diet. FCR values, calculated for 0 and 1000 FTU kg^{-1} level diets were greatly different (P<0.05) while reference, 500, 1500, 2000 diets were significantly same as shown in Table 3. Analysed nutrient (crude protein, crude fat and apparent gross energy) contents of reference and test diets are demonstrated in Table 4 and fecal matter in Table 5. Nutrients digestibility (%) for soybean meal based test diets are shown in Table 6. Our results showed that test diets with 1000 and 1500 FTU kg^{-1} levels of phytase enzyme in comparison with reference diet and rest of soybean meal based diets, cause slightest amount of nutrients loosed by feces (Table 5). ANOVA on the data shows that, it is superficial that maximum apparent protein digestibility (%) was observed at 1000 and 1500 FTU kg^{-1}. The highest apparent fat as well as digestibility values of gross energy in soybean meal based test diet noticed at 1000 FTU kg^{-1} level and the next higher digestibility value was observed at 1500 FTU kg^{-1} level. It was observed that except 1000 and 1500 FTU kg^{-1} levels, as compared to the reference diet all other remaining levels of phytase supplementation in soybean meal diets did not show prominent crude protein digestibility. Crude fat and apparent gross energy digestibility also resulted best at 1000 FTU kg^{-1} followed by 1500 FTU kg^{-1} level, while maximum digestibility of crude protein was observed at 1000 FTU kg^{-1} level. Feeds treated with further higher levels of phytase enzyme resulted in drastic decrease in crude protein digestibility.

Table 1. Chemical composition (%) of feed ingredient

Ingredients	Dry matter (%)	Crude Protein (%)	Crude Fat (%)	Crude fiber (%)	Ash (%)	Gross Energy (kcalg¹)	Carbohydrates
Fish meal	91.63	48.15	7.16	1.07	26.73	2.69	16.89
Wheat flour	92.45	10.10	2.35	2.65	2.08	2.96	82.82
Corn gluten 60%	92.33	59.48	4.56	1.19	1.39	4.32	29.06
Rice polish	94.09	12.35	13.54	12.70	10.18	3.33	51.23
Soybean meal (Ingredient)	93.80	41.93	3.74	1.97	10.83	3.54	37.99

Discussion

Results of present work give us clear indication that growth, weight gain, minerals and nutrient digestibility as well as FCR of *Cirrhinus mrigala* fingerlings are maximally increased with the soybean meal based diet supplemented by phytase to a level of 1000 FTU kg^{-1} followed by diet supplemented with phytase at 1500 FTU kg^{-1} level. The findings of the current study provided signal that the level of 1000 FTU kg^{-1} diet was sufficient for reducing the phytic acid effects and to release the chelated minerals and protein of plant based diets (soybean). Present results about growth performance of *C. mrigala* on soybean meal based diets are comparable with the findings of Baruah *et al.* (2007a) and Hussain *et al.* (2011). Their findings conclude improved performance of *L. rohita*

fingerlings when fish fed on plant based diets supplemented with phytase. Liebert and Portz (2005 & 2007) also give comparable results reported in Nile tilapia (*Oreochromis niloticus*) with phytase supplemented plant based diet (SP 1002). Many researchers, using plant based diets in aquaculture found optimum level for phytase be 1000 FTU kg^{-1}(Riche and Garling, 2004; Ashraf and Goda, 2007; Cao *et al.,* 2008). Carnivorous species like catfish (*Ictulurus punctutus*) and Atlantic salmon opposes the present findings and showed no significant change in weight gain after the use of phytase supplemented plant based diets (Yan and Reigh, 2002; Sajjadi and Carter, 2004).

Table 2. Ingredients composition (%) of reference and soybean meal based test diets.

Ingredients	Reference diet	Test diets
Fish meal	20.0	14.0
Wheat flour	24.0	15.9
Corn gluten 60%	20.0	14.0
Rice polish	25.0	17.5
Fish oil	7.0	4.9
Vitamin Premix	1.0	1.0
Minerals	1.0	1.0
Ascorbic acid	1.0	1.0
Chromic oxide	1.0	0.7
Soybean meal (Test ingredient)	-	30.0
Total	100.0	100.0

Table 3. Growth performance of *Cirrhinus mrigala* fingerlings fed on reference and phytase supplemented soybean meal-based test diets.

Parameters	Reference diet	Test diet-I	Test diet-II	Test diet-III	Test diet-IV	Test diet-V
		Phytase levels (FTU kg^{-1})				
		0	500	1000	1500	2000
Initial weight (g)	7.06±0.02	7.05±0.01	7.03±0.02	7.05±0.010	7.05±0.02	7.05±0.01
Final weight (g)	15.46±0.30	14.39±0.46	16.20±0.32	17.98±0.33	16.99±0.50	15.90±0.31
Weight gain (g)	8.40±0.30^{c}	7.35±0.46^{d}	9.17±0.30b^{c}	10.93±0.34^{a}	9.94±0.49^{ab}	8.85±0.32^{c}
Weight gain (%)	118.97±4.17^{c}	104.21±6.63^{d}	130.3±4.02b^{c}	155.04±5.05^{a}	140.96±6.70^{ab}	125.58±4.67^{c}
Weight gain(fish^{-1} day^{-1}) g	0.12±0.01	0.10±0.01	0.13±0.01	0.15±0.01	0.14±0.01	0.12±0.01
Feed intake (fish^{-1} day^{-1})g	0.20±0.01	0.18±0.01	0.22±0.01	0.22±0.01	0.21±0.01	0.21±0.01
FCR	1.70±0.09^{ab}	1.74±0.11^{a}	1.68±0.14^{ab}	1.43±0.08^{b}	1.51±0.02^{ab}	1.69±0.14^{ab}

Means within rows having different superscripts are significantly different at P < 0.05.

Highest crude protein digestibility percentage (%) of *Cirrhinus mrigala* fingerlings fed on soybean meal based test diet was observed with 1000 FTU kg^{-1} diet. Findings of other researchers like Baruah *et al.* (2007a) and Liebert and Portz, (2007) favours the present results though little data is available for comparison (Vielma *et al.,* 2004; Cao *et al.,* 2007 Laining *et al.,* 2010; Nahashon and Agnes, 2013). Current work showed the higher apparent digestibility coefficient (ADC) for apparent protein with soybean meal based diet supplemented by phytase enzyme at1000 FTU kg^{-1}. Similar results were reported by Vielma *et al.* (2004), Liebert and Portz, (2005), Ashraf and Goda (2007), Nwanna *et al.* (2008), Wang *et al.* (2009), Laining *et al.* (2011) as well as Olusola and Nwanna (2014). Contrarily, Yan and Reigh (2002), Sajjadi and Carter (2004) and Dalsgaard *et al.* (2009) did not observe any substantial effect on digestibility of protein in fish giving phytase supplemented diet. Oppositely Hossain and Jauncey (1993) and Teskeredzic *et al.* (1995) reported decline in digestibility of protein by

supplemented phytase diet. This deviation, observed in a number of studies for digestibility of nutrient, may be linked to protein quality of feed, pH of fish stomach and procedures used for drying (Wang *et al.,* 2009). Generally, the impact of supplementation of phytase on nutrient digestibility depend on a variety of factors such as source of phytate, concentration and protein sources in the alternative diet (Shao *et al.,* 2008) and protein source digestibility (Liu *et al.,* 2013).

Table 4. Analyzed compositions (%) of apparent crude protein, apparent crude fat and gross energy in the diet of *Cirrhinus mrigala* fingerlings fed on reference and soybean meal based test diets

Experimental diets	Phytase levels(FTUkg^{-1})	Apparent crude protein (%)	Apparent crude fat (%)	Apparent gross energy (%)
Reference Diet	---	32.06±0.015	6.60±0.025	4.87±0.015
Test Diet- I	0	31.31 ±0.035	5.56±0.080	4.24±0.015
Test Diet -II	500	31.32±0.020	5.56±0.015	4.25±0.011
Test Diet -III	1000	31.30±0.037	5.56±0.015	4.24±0.015
Test Diet- IV	1500	31.31±0.011	5.56±0.025	4.24±0.020
Test Diet- V	2000	31.31±0.036	5.57±0.005	4.25±0.015

Table 5. Analyzed compositions (%) of apparent crude protein, apparent crude fat and gross energy in the feces of *Cirrhinus mrigala* fingerlings fed on reference and soybean meal based test diets.

Experimental diets	Phytase levels (FTU kg $^{-1}$)	Apparent crude protein (%)	Apparent crude fat (%)	Apparent gross energy (%)
Reference Diet	---	15.32±0.319	2.98±0.122	2.18±0.130
Test Diet- I	0	15.06±0.250	2.74±0.045	1.94±0.06
Test Diet -II	500	13.65±0.421	2.15±0.064	1.74±0.090
Test Diet -III	1000	9.32±0.744	2.09±0.266	1.76±0.05
Test Diet- IV	1500	12.15±0.270	2.15±0.096	2.05±0.07
Test Diet- V	2000	31.31±0.036	2.79±0.147	2.10±0.10

Similarly highest values of gross energy digestibility and apparent fat digestibility were observed at 1000 FTU kg^{-1} diet. Different researchers (Portz and Liebert, 2004; Ashraf and Goda, 2007) found similar level or a little bit higher doses (1000-2000 FTU kg^{-1}) of phytase effective when supplemented in diet. In contrary, the doses of phytase above 1000 FTU kg^{-1}caused significant decline in digestibility coefficients of fat due to limited amount of fat in diets used in experiment (Wang *et al.,*2009). But no effect was observed on the apparent fat and digestibility of gross energy (Dalsgaard *et al.,* 2009).In present research work, phytase supplementation improved digestibility in gross energy.

Table 6. Apparent nutrient digestibility (%) of soybean meal ingredient based diet.

Experimental diets	Phytase levels (FTU kg $^{-1}$)	Protein (%)	Fat (%)	Gross Energy (%)
Reference Diet	---	56.62±1.29^{bc}	59.95±0.16^{cd}	57.94±1.38^{c}
Test Diet- I	0	53.14±1.73^{c}	52.01±1.55^{e}	55.59±0.91^{c}
Test Diet -II	500	60.02±2.30^{b}	64.39±2.31^{bc}	62.57±0.33^{b}
Test Diet- III	1000	70.66±1.65^{a}	70.91±1.57^{a}	67.80±1.99^{a}
Test Diet -IV	1500	66.74±2.40^{a}	66.89±2.36^{ab}	58.74±0.68^{c}
Test Diet- V	2000	57.98±2.47^{bc}	56.77±0.95^{d}	57.37±1.16^{c}

Means within rows having different superscripts are significantly different at $P < 0.05$.

The irregularity in the behaviour of phytase action by different researchers may be due to differences in type of phytic acid and contents in unlike feed ingredients, nutritional quality of ingredients, fish

species, water parameters and quality in addition with size as well as conditions used in experiments (Ashraf and Goda, 2007). Moreover, during diet manufacturing method that was used for phytase addition, such as pre-treatment of feed ingredient, may also have impacts on utilization efficiency of feed and growth performance in fish (Ashraf and Goda, 2007; Liu *et al.*, 2013; Olusola and Nwanna 2014).

Plant based diets like soybean meal supplemented by phytase enzyme at the graded level 1000 FTU kg^{-1} to 1500 FTU kg^{-1}, enhanced the growth performance and nutrient digestibility of *Cirrhinus mrigala* and further research is needed to explore efficacy of phytase to design better, nutrient rich and environment friendly cheap alternate fish feed sources.

Acknowledgement

The authors are grateful to Higher Education Commission, Islamabad, Pakistan for financial support by funding project No. PM-IPFP/HRO/HEC/2011/ and GC University, Faisalabad for facilitating this research work.

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