



Effect of Acidification in a Phytase Sprayed Sunflower Meal Based Diet on Growth and Dietary Nutrient Digestibility Performances of *Labeo rohita* Juveniles

Muhammad Afzal,^{1*} Ammara Amanat,¹ Syed Zakir Hussain Shah¹ and Syed Makhdoom Hussain²

¹Fish Nutrition Laboratory, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan

²Department of Zoology, Government College University, Faisalabad, Pakistan

ABSTRACT

An experiment was conducted to investigate the effect of citric acid supplementation on a diet consisting of sunflower meal as major protein source in *L. rohita* juveniles. Three experimental diets were made by supplementing phytase at constant level (750 FTU/kg) while citric acid was added at the level of 0%, 1% and 2% in the basal diet resulting in the formation of three experimental diets designated as SFM₁, SFM₂ and SFM₃. Chromic oxide (1%) was added as an inert marker to all experimental diets to measure the dietary nutrient digestibility. Growth and digestibility performances of juveniles showed significant ($p < 0.05$) responses against diet acidification. Maximally improved ($p < 0.05$) weight gain and feed conversion ratio were observed in SFM₃ diet supplemented with 2% citric acid. Likewise, maximum apparent dietary nutrient digestibility was also recorded in SFM₃ diet. In conclusion, acidification of sunflower meal based diet with citric acid improved the growth performance and nutrient digestibility in *L. rohita* juveniles. Further research is needed to investigate higher levels of acidification for any additional improvement in nutrient digestibility and growth performance.

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Authors' Contribution

MA conceived and designed the study. AA conducted the experiment. AA, SZHS and SMH analyzed the data. SZHS wrote the article.

Key words

Citric acid, Phytase, *L. rohita*, sunflower meal, growth performance, nutrient digestibility

INTRODUCTION

Carp species including *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* are being cultivated throughout the Indian subcontinent and contribute about 87% of the total freshwater aquaculture production (ICLARM, 2001). Among these carp species, *L. rohita* has high market value and is being cultured semi-intensively and intensively. However, intensive aquaculture practice required cost effective and low polluting fish feed. In these days, practice of including plant protein sources in *L. rohita* feed is increasing due to their low cost and plentiful availability. Herbivorous feeding habit of *L. rohita* provides another advantage to these plant protein based diets. Among these plant based meals, sunflower meal is considered the most appropriate and promising protein source as it contains high protein while low phosphorus (P) contents and is easily available as compared to fish meal (Hardy, 1995). Nevertheless, these plant based protein constituents have anti-nutritional factors like phytate which interfere with the normal process of nutrient absorption resulting in enhanced waste production (Hardy, 1996) leading to environmental pollution. It also chelates essential minerals including

Ca, P, Mg, Na, K, Fe, Cu and Zn and protein and reduces their availability to the fish resulting in stunted growth (Papatryphon *et al.*, 1999; Sugiura *et al.*, 2001).

Organic acids are promising supplements in plant based feeds for phytate hydrolysis. Zyla *et al.* (1995) found in an *in vitro* study that citric acid intensifies the phytate dephosphorylation. Phytate hydrolysis results in release of bound minerals and other nutrients which ultimately become available to the fish leading to improved growth performance. Previous studies indicated improved growth and feed performance of *L. rohita* (Baruah *et al.*, 2007), *Cyprinus carpio* (Khajepour *et al.*, 2012) and *Huso huso* (Khajepour and Hosseini, 2012) in response to citric acid supplementation in plant meal based diets.

Phytase is an enzyme chemically known as myo-inositol hexaphosphate phosphohydrolase (Class 3: Hydrolases), catalysis the breakdown of phytate resulting in release of bound nutrients (Nagai and Funahashi, 1962). It has been reported in several studies that phytase supplementation to plant meal based diets improved the nutrient digestibility resulting in improved growth performance (Nwana *et al.*, 2007, 2008; Sardar *et al.*, 2007). Moreover, our previous studies showed that phytase addition to plant based diets improved growth performance and nutrient utilization in *L. rohita* juveniles (Hussain *et al.*, 2011a,b,c; 2015). Its activity varies in different parts of the gut due to change in pH (Yi and Kornegay, 1996), whereas, its optimum activity was

* Corresponding author: afzal160@uaf.edu.pk

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recorded at pH 2.5 and 5.0-5.5 (Simons *et al.*, 1990). However, in agastric (stomachless) fishes like *L. rohita*, due to lack of acid secretion in the gut, pH remains above 6. Hence, it is hypothesized that addition of organic acid in the phytase sprayed *L. rohita* diet may lower the gastric pH and provide optimum conditions for phytase functioning. Therefore, present study was designed to determine the effect of acidification in a phytase treated diet by evaluating the growth performance and nutrient digestibility of *L. rohita* juveniles.

MATERIALS AND METHODS

Diets and experimental design

The experimental diets were formulated using sunflower meal (SFM) as a major protein source (Table I). Feed ingredients were obtained from local poultry feed market, Faisalabad. Citric acid was supplemented at the level of 0%, 1% and 2% resulting in the formulation of three experimental diets designated as SFM₁ (control diet), SFM₂ and SFM₃. Chromic oxide (1%) was added as an inert marker to all experimental diets to measure the dietary nutrient digestibility. Ingredients were ground and dough was made by adding appropriate amount of water to mix the ingredients. Pellets of 2 mm size were made by using Lab Extruder (model SYSLG30-IV). Phytase (Phyzyme ® XP 10000 FTU/g; Danisco Animal Nutrition, Fin-65101 Vaasa, Finland) was sprayed on pellets of all experimental diets at a constant level of 750 FTU/kg. Thereafter, feed was stored in vacuum-packed bags and kept frozen (-18°C) until use.

Fish, experimental conditions and feeding

Fish juveniles were obtained from Government Fish Seed Hatchery, Faisalabad and distributed to nine experimental tanks having three replicates for each experimental diet. Fifteen juveniles (Average weight=4.04±0.004) were distributed in each tank (70 L capacity) randomly. Continuous aeration was provided to all experimental tanks throughout the experimental period. Important water quality parameters including temperature, pH, dissolved oxygen (DO) and salinity were monitored daily. Juveniles were fed twice daily at 2% of body weight, over 8 weeks.

Growth studies

Initial weight was measured at the start of the experiment and growth and feed performance were measured by following standard formulae.

$$\text{Weight gain} = \text{Mean final weight} - \text{Mean initial weight}$$

$$\text{Weight gain \%} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{FCR} = \frac{\text{Dry feed fed}}{\text{Weight gain}}$$

Table I.- Ingredients and proximate composition of experimental diets.

Ingredients (%)	SFM1 (Control diet)	SFM2	SFM3
Fish meal	5	5	5
Wheat flour	5	5	5
Corn gluten 60%	5	5	5
Rice polish	8	8	8
Fish oil	3	3	3
Vitamin premix*	1	1	1
Mineral mixture**	1	1	1
Ascorbic acid	1	1	1
Chromic oxide	1	1	1
Sunflower meal	70	70	70
Phytase (FTU/kg)	750	750	750
Citric acid (%)	0	1	2
Proximate analysis			
Dry matter (%)	98.56±0.04	98.21±0.02	97.93±0.01
Crude protein (%)	26.88±4.17	29.99±0.06	30.06±0.04
Crude fat (%)	3.28±0.01	3.25±0.02	3.3±0.01

*Each kg of vitamin premix contains Vitamin A, 15 M.I.U.; Vitamin D₃, 3 M.I.U.; Vitamin B₁, 5000 mg; Vitamin E, 6000 IU; Vitamin B₂, 6000 mg; Vitamin K₃, 4000 mg; Vitamin B₆, 4000 mg; Folic acid, 750 mg; Vitamin B₁₂, 9000 mg; Calcium pantothenate, 10000 mg; Vitamin C, 15000 mg; Nicotinic acid, 25000mg

**Each kg of mineral mixture contains Ca, 155gm; Mn, 2000mg; P, 135gm; Cu, 600mg; Mg, 55gm; Co, 40mg; Fe, 1000 mg; I, 40mg; Zn, 3000 mg; Se, 3mg; Na, 45gm

Digestibility studies

Fecal material was collected as described previously (Hussain *et al.* 2011abc). Samples were oven dried and finely grounded to analyze the chemical composition. Chromic oxide concentration of feed and feces samples was measured as described by Furukawa and Tsukahara (1966). Proximate composition including dry matter, crude protein, crude fat and gross energy of ingredients, feed and feces samples were measured following AOAC (1995). Apparent nutrient digestibility coefficients were determined by using the following 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

Data were subjected to one-way analysis of variance (ANOVA). When statistical differences (p<0.05) were observed, Tukey's honesty significant difference test was used to compare the group means. Costat (version 6.303) software was used to perform all statistical analysis.

Table II.- Effect of citric acid acidification in a phytase treated SFM based diet on the growth performance of *L. rohita* fingerlings.

Experimental diets	Initial weight (g)	Final weight (g)	Weight gain (g)	Weight gain (%)	FCR
SFM ₁ (Control diet)	4.05±0.007	7.69±0.14	3.63±0.13 ^c	89.620±3.13 ^c	2.21±0.03 ^c
SFM ₂	4.04±0.004	8.21±0.08	4.17±0.84 ^b	103.09±2.27 ^b	1.82±0.04 ^b
SFM ₃	4.06±0.007	8.94±0.08	4.87±0.08 ^a	119.91±1.71 ^a	1.32±0.05 ^a

Data are means of 3 replicates ± SE

Mean values of same parameter sharing different superscripts vary significantly (p<0.05)

RESULTS

The data of fish growth and feed performance influenced by the acidification of phytase sprayed sunflower meal based extruded feed is presented in Table II. The growth performances data were significantly (p<0.05) affected by acidification of diet. Highest weight gain (%) (p<0.05) was recorded in fish fed SFM₃ diet supplemented with 2% citric acid. Feed conversion ratio (FCR) was also significantly (p<0.05) affected by citric acid addition to the diet. Highest FCR was recorded in group fed on diet containing 2% citric acid supplementation which also varied significantly (p<0.05) from all other groups.

Effect of citric acid acidification in a phytase treated diet on digestibility of dietary nutrients has been presented in Table III. Nutrients digestibility was significantly affected by citric acid supplementation (p<0.05). Maximum digestibility (p<0.05) of crude protein was observed in SFM₃ diet having 2% citric acid supplementation. Addition of 1% citric acid in the diet (SFM₁) also resulted in significant enhancement of crude protein digestibility in comparison of control group. Likewise, crude fat and gross energy digestibility also followed the same trend as that was observed in the case of crude protein digestibility.

Table III.- Effect of citric acid acidification in a phytase treated SFM based diet on the digestibility performance of nutrients in *L. rohita* fingerlings

Experimental diets	Crude protein (%)	Crude fat (%)	Gross energy (%)
SFM ₁ (Control diet)	44.76±0.91 ^c	47.13±0.58 ^c	43.04±1.54 ^c
SFM ₂	53.29±1.42 ^b	55.14±2.11 ^b	48.26±0.94 ^b
SFM ₃	63.2±0.98 ^a	65.3±1.52 ^a	56.11±0.97 ^a

Data are means of 3 replicates ± SE

Mean values of same parameter sharing different superscripts vary significantly (p<0.05)

DISCUSSION

The present study showed improved growth performance of *L. rohita* fingerlings in response to addition of citric acid in phytase treated SFM based diet. Highest weight gain was recorded in phytase treated SFM₃ diet supplemented with 2% citric acid. Inclusion of organic acids in the diet might resulted in decrease of diet pH which lead to reduced intestinal pH. Lower pH in gastrointestinal tract causes improved absorption of nutrients (Boling *et al.*, 2001). It also causes death of harmful micro-biota and favors the growth of beneficial micro-organisms which ultimately results in improved growth performance of fish (Ferd, 1974). It has also been reported that citric acid itself intensifies the phytate dephosphorylation (Baruah *et al.*, 2007) which resulted in release of bound minerals and nutrients which contributed in the improvement of growth. Furthermore, citric acid might have provided a suitable environment to the microbial phytase to liberate enough mineral and other nutrients from phytate which led to significantly increased growth performance of fish.

In agreement to our study, improved (p<0.05) weight was also reported by citric acid addition (1%) in Red sea bream, *Pagrus major* (Hossain *et al.*, 2007; Sarker *et al.*, 2007) and Yellowtail, *Seriola quinqueradiata* (Sarker *et al.*, 2012). A similar increase (p<0.05) in weight gain (%) was also reported by Phromkunthong *et al.* (2010) in common carp, *Cyprinus carpio* having citric acid acidified phytase treated diet. Baruah *et al.* (2007) also reported significantly (p<0.001) enhanced weight gain (%) in plant meal based diet supplemented with 3% citric acid to a phytase level of 500 FTU/kg.

Dietary citric acid inclusion in phytase treated diet in the current study also showed significantly (p<0.05) improved feed conversion ratio. Other studies have also reported improved feed conversion rate in Beluga, *Huso huso* (Khajepour and Hosseini, 2012), Rohu, *Labeo rohita* (Baruah *et al.*, 2007) and Common carp, *Cyprinus carpio* (Khajepour *et al.*, 2012) in a citric acid (3%)

supplemented diet. Improved feed conversion ratio has also been reported with the addition of citric acid and phytase in the diet of common carp (Phromkunthong *et al.*, 2010).

Apparent digestibility of gross energy, crude protein and crude fat observed in the present study was significantly improved by acidifying the phytase containing sunflower meal based diet in *L. rohita* juveniles. It has also been observed that by increasing the citric acid level from 1% to 2% in the fish diet also significantly enhanced the digestibility of these parameters. Partanen and Mroz (1999) advocated that nutrients digestibility is directly influenced by the level of organic acid used. In contrary to our findings, no significant change was observed in the digestibility of crude protein, however, crude fat digestibility was significantly ($p < 0.05$) enhanced in rohu fingerlings having citric acid supplemented diet (Baruah *et al.*, 2007).

CONCLUSION

In conclusion, acidification of sunflower meal based diet with citric acid improved the growth performance and nutrient digestibility in *L. rohita* juveniles.

Statement of conflict of interest

Authors have declared no conflict of interest.

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