INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
16–835/2017/19–2–363–366
DOI: 10.17957/IJAB/15.0292
http://www.fspublishers.org

Short Communication

The Potential Use of Citric Acid for the Improvement in Growth of *Labeo rohita*

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Abstract

Fish nutritionist have been working to formulate a fish feed with less pollution causing agents. Plants by-products have been used as alternative to fish meal for both the purposes and experiments remain successful for some fish species. The present study was aimed to determine the sunflower meal suitability with citric acid supplementation for *Labeo rohita* by observing growth parameters. Three diets were prepared as 0, 15 and 30 g/kg citric acid. The fish were fed with experimental diets for two months. Results showed that the fish fed 0% citric acid had low phosphorus and calcium digestibility than that fed higher levels 15 and 30 g/kg of citric acid ($p < 0.05$). The growth performance of *L. rohita* was higher at the 30 g/kg supplementation level of citric acid and decreasing trend was observed with the decreasing level of citric acid. The above results indicated that the supplementation of CA could substitute the inclusion of inorganic phosphorus in *L. rohita* diets and the supplementation level was suggested to be 30 g/kg.

Keywords: *Labeo rohita*; Citric acid; Phosphorus digestibility; Growth performance

Introduction

Formulated diets contains a sufficient amount of phosphorus in fish diet but a large amount of it is in the form of phytate in plants by-products like sunflower meal and in the form of tri-calcium phosphate in fish meal. These both tri-calcium phosphate and phytate cannot be utilized by *Labeo rohita*. To fulfill the requirement of fish, phosphorus is added in the feed as inorganic phosphate but this may also increase the excretion of phosphorus which can pollute the environment. Improvement in phosphorus utilization and reduction of discharge by reducing phosphorus inclusion remained an important area of nutritional research. Exogenous enzymes are considered important in nutrients as well as phosphorus utilization in different fish species like trout (*Oncorhynchus mykiss*) (Vandenberg *et al*., 2011), sea bream (*Pagrus major*) (Laining *et al*., 2012) and herbivore fish (*Ctenopharyngodon idellus*) (Liu *et al*., 2013). Citric acid is among organic acids which is reported to advance the digestion with energy metabolism in poultry and growth and health in mammals by making the phosphorus and nutrients available and inhibiting the pathogens (Castillo *et al*., 2004; Giannenas, 2006). Now a day's citric acid has gained importance for its activity in the enhancement of feed utilization and health of fish and shellfish diets (Lim *et al*., 2010; Silva *et al*., 2013).

Citric acid is famous in pharmaceutical and food industries for flavoring and buffering activity. Citric acid as acidifier is reported to improve digestibility, growth and relieve stress in birds (Agustın *et al*., 2003; Gauthier, 2005; Ao *et al*., 2009; Islam *et al*., 2012), pig (Partanen and Mroz, 1999; Narayanan *et al*., 2008) and aquatic animals like in drum (*Sciaenops ocellatus)*, where citric acid enhanced the digestive enzymes activities (Castillo *et al*., 2014), In rainbow trout acidification of short fish meal feed improved the growth and P retention (Adrian *et al*., 2012). The same improvement in growth and nutrients absorption by citric acid was correspondingly testified in tilapia (*Oreochromis niloticus* × *O. aureus*) (Pan *et al*., 2004), sea bream (Sarker *et al*., 2005), sturgeon (Khajepour and Hosseini, 2012), rainbow trout (Sugiura *et al*., 1998; Vielma *et al*., 1999; Pandey and Satoh, 2008), crucian carp (*Carassius auratus*, Gibelio) (Leng *et al*., 2006), and white shrimp (*Litopenaeus vannamei*) (Su *et al*., 2014). Moreover, citric acid (5–30 g/kg) improved phosphorus utilization in sea bream (Sarker *et al*., 2005; Hossain *et al*., 2007), improved bioavailability of minerals in sturgeon (Khajepour and Hosseini, 2012).

The improvement in utilization and digestibility of P by citric acid, provoked towards the substitution of the inanimate phosphate in diet. Even though by a number of experiments, the sound properties of citric acid in fish diet without inorganic phosphate supplementation have been studied (Pandey and Satoh, 2008; Adrian *et al*., 2012). The citric acid levels in those experiments did not clearly indicate that how much citric acid could be used in the diet of fish.

To cite this paper: Bano, N., M. Afzal, S. Abdullah and M. Shahid, 2017. The potential use of citric acid for the improvement in growth of *Labeo rohita*. *Int. J. Agric. Biol*., 19: 363–366

Rohu is famous in India and Pakistan for its taste. However, its production is low because of massive death of the fish at fingerling stage due to unsuitable nutrition. Therefore, the objective of this study is to find out the appropriate citric acid level in *L. rohita* diet, by evaluating the effects of CA on growth, calcium and P digestibility and body composition of *L. rohita*.

Materials and Methods

Experimental Details

In this factorial experiment, three citric acid levels (0, 15 and 30 g/kg) were used. This was a completely randomized experiment with three replications of each treatment. The ingredients and chemical composition of diets is shown in Table 1 and 2. All the ingredients of diet were mixed in a mixer, moistened by adding distilled water and pellets made using extruder. The pellets were then dried at room temperature and refrigerated at 4˚C till the start of feeding trial.

Growth Trial

A total of 150 fish were selected from the fish seed hatchery Faisalabad (Punjab, Pakistan) and supplied in fish nutrition laboratory, department of Zoology, Wild life and Fisheries, University of Agriculture, Faisalabad, Pakistan. Formerly, all fish were acclimatized to laboratory conditions for 15 days and fed with the basal experimental diet 1 (Table 1). All fish were famished for 24 h before the start of feeding trial. A total of 108 fish of similar size (~3.4 g) were disseminated in 9 steel tanks (70 L) with 12 fish in each tank. Drainage pipes were used for the changing medium from tanks. Which was done twice a day. Triplicate tanks (v shape at base) were used for each treatment with aerators. The feeding trial lasted for 60 days. During all experiment, the water temperature was 26.5±0.3˚C, dissolved oxygen 6.7±0.05 mg/L, pH 7.4±0.04 and electric conductivity remain 1.32-1.53 dS/m. The fish were fed the experimental diet at 08:00 am for two months. Uneaten feed was collected after an hour of feeding and oven dried at 60˚C for feed consumption analysis. The fecal material was collected carefully after 2 h of feeding to avoid leaching of calcium and phosphorus from the base of tank by opening the valve at the base of tank. The fecal material was oven dried at 60˚C for further analysis.

Sample Collection and Analysis

Initial fish weight was noted at the start of the research trial. After the completion of nurturing trial, the fish were weighed to determine the growth parameters. Chemical composition of prepared diets and feces was determined as: Dry matter by constant heating at 105˚C for 6 h and crude protein by Kjeldahl method (AOAC, 2005). Gross energy was calculated by oxygen bomb calorimeter and Crude lipid by extraction with petroleum ether for 12 h in a Soxhlet extractor (AOAC, 2005). Mineral contents of test diets and feces were determined by AOAC (2005) by using mixture of nitric acid and perchloric acid (2:1) and by means of Atomic Absorption Spectrophotometer (Hitachi Polarized Atomic Absorption Spectrometer, Z-8200) as recommended by manufacturers.

Calculation and Statistical Analysis

The percent weight gain, FCR, SGR and apparent digestibility coefficients were calculated by using following equations:

Weight gain (%) = 100 × (final weight – initial weight) / initial weight.

Specific growth rate (%) = 100 × (In average final weight – In average initial weight) / duration of experiment in days.

Feed conversion ratio (FCR) = feed intake/absolute weight gain.

Apparent digestibility of nutrients (%) = 100 - 100 × [percent chromic oxide in diet × percent nutrient in feces / percent chromic oxide in feces × percent nutrient in diet].

Mean values of three replicate are described ± standard error. After indorsing the homogeneity of variance and normality, data was analyzed by one way ANOVA and differences were considered significant at P<0.05 using Costate (6.303, PMB320, Monterey, CA, 93940 USA). The linear regression was used to see the relation between citric acid and weight gain.

Results

Fish Growth

The data of growth for *L. rohita* in terms of weight gain (g), percent weight gain, feed conversion ratio (FCR) and specific growth rate (SGR) ware found to be significantly different (p<0.05). After two months feeding trial, the lowliest growth observed was in group nourished with diet containing 0% citric acid. When compared with other diets, 15 and 3 g/kg citric acid significantly improved the growth of fish. There was a decrease in weight gain with the increase in FCR. The maximum weight gain 2.90 g was found to be at 30 g/kg citric acid supplementation level (Table 3). There was a linear relation between weight gain and citric acid level as: Y= 0.03x + 2.81 (R^2 = 1).

Calcium and Phosphorus Digestibility

Data revealed an increase in digestibility of calcium and phosphorus with an increase in the levels of citric acid. There was significant difference (p<0.05) among all the diets and the higher digestibility of calcium (63%) and phosphorus (73%) was observed in the group fed on 30 g/kg citric acid supplemented diet (Table 4).

Table 1: Formulation of the experimental diets

Ingredients	C_0	$C_{1.5}$	C_3
Fish meal[a]	51	51	51
Sunflower meal[a]	16	16	16
Starch	10	10	10
Rice polish[a]	16	16	16
fish oil	3	3	3
Vitamin premix[b]	1	1	1
Mineral premix[b]	1	1	1
Ascorbic acid	1	1	1
Chromic oxide	1	1	1
Citric acid	0	1.5	3

[a]fish meal (% dry matter): crude protein23.4, crude lipid0.9, ash13.6 ; sunflower meal (% dry matter): crude protein 6, crude lipid2.2, ash1.05; starch (% dry matter): crude protein 1.2, crude lipid 0.25, ash 0.17; rice polish (%dry matter): crude protein 1.76, crude lipid 2.1, ash 1.6
[b]vitamin premix: same as Hussain *et al.* 2015b

Table 2: Proximate and mineral composition of the experimental diets

diets	C_0	$C_{1.5}$	C_3
Dry matter (%)	94.14	94.25	94.43
Crude protein (%)	32.42	32.22	32.24
Crude fat (%)	10.11	10.92	10.92
Gross energy (kcal/g)	2.54	2.57	2.53
P (%)	2.60	2.63	3.15
Ca (%)	3.27	3.68	3.81
Mg (%)	0.82	0.85	0.92
K (%)	0.60	0.61	0.98
Na (%)	0.62	0.64	0.99
Mn (%)	0.003	0.004	0.004
Zn (%)	0.009	0.011	0.012
Cu (%)	0.001	0.001	0.001
Fe (%)	0.024	0.025	0.025

Table 3: Growth performance of *L. rohita*

Diets	Initial weight	Final weight	Weight gain (g)	Weight gain (%)	FCR	SGR (%)
C_0	3.28	6.82±0.07	3.54±0.07^c	107.9±1.91^c	2.36±0.01^a	0.99±0.02^e
$C_{1.5}$	3.26	6.83±0.05	3.57±0.08^b	109.5±0.50^b	1.98±0.02^b	1.01±0.01^e
C_3	3.26	6.86±0.04	3.60±0.06^a	110.4±1.26^a	1.89±1.16^b	1.01±0.01^e

Mean ± standard error. Values with different letters differ significantly (P<0.05)

Table 4: Calcium and phosphorus apparent digestibility coefficient

diets	Calcium (%)	Phosphorus (%)
C_0	58.18±0.45^c	58.20±0.21^c
$C_{1.5}$	61.03±0.27^b	59.40±0.34^b
C_3	64.83±0.11^a	75.05±0.44^a

Mean ± standard error. Values with different letters differ significantly (P<0.05)

Discussion

The supplementation of citric acid have been proved by different studies as in rainbow trout (Adrian *et al*., 2012), sturgeon (Khajepour and Hosseini, 2012) and red drum (Castillo *et al*., 2014). In this present work citric acid 30 g/kg also have improved the growth rate and reduce the FCR in *Labeo rohita.* It is important to note that the level mentioned are different in different experiments (Adrian *et al*., 2012). The level of citric acid was 30 g/kg for sturgeon (Khajepour and Hosseini, 2012) but 15 g/kg for red drum (Castillo *et al*., 2014) and 10 g/kg in trout (Adrian *et al*., 2012).

Higher levels of citric acid may disrupt the activity of digestive tract by unbalancing the pH. The differences in the levels of citric acid depends on the mode of feeding, habitat of fish, and the type of digestive tract (according to species). It is common belief that carnivorous fish having stomach for example rainbow trout can tolerate higher levels of citric acid as compare to the agastric fish like *L. rohita*.

The calcium and phosphorus digestibility was observed to be improved by the supplementation of 30 g/kg citric acid in *L. rohita*. Citric acid not only increased the phosphorus and calcium utilization in fish but also in pigs by improving their absorption in small intestine (Zhao *et al*., 2011). Citric acid decreased the pH in feed and gut of Indian major carps (Baruah *et al*., 2005). However, in red drum it did not affect the intestinal pH but stomach pH was reduced. Therefore, a main reason for increment in phosphorus and calcium absorption is other than the reduction in pH at intestine (Castillo *et al*., 2014). Acidity at the stomach level in digestive tract, positively effects the absorption of minerals (Hasan and Khan, 2013) as it regulates the binding process of minerals and nutrients as well as complex formation (Hien *et al*., 2015). A lower pH positively influenced the conversion of pepsinogen to pepsin as pepsin works better in 2 to 3 pH range (Zhao *et al*., 2011).

Conclusion

Citric acid in diet of *L. rohita* improved the health, growth performance, digestibility of calcium and phosphorus by decreasing the pH in digestive tract. The suggested citric acid supplementation level for *L. rohita* was 30 g/kg.

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(Received 10 December 2016; Accepted 14 February 2017)