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Effect of citric acid supplementation on growth parameters of *Cirrhinus mrigala* fingerlings fed canola meal based test diets

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Abstract

Increasing demand with high price and unstable supply of the fish meal with the expansion of aquaculture made it necessary to search alternative protein sources. Due to higher pH of digestive tract, fish cannot absorb nutrients. Use of citric acid is beneficial for the absorption of nutrients by decreasing pH of digestive tract. Therefore, the aim of the current study was to explore the effect of citric acid supplementation on growth performance of *Cirrhinus mrigala* fingerlings fed canola meal based test diets. Six experimental test diets were formulated by supplementing with graded levels (0%, 1%, 2%, 3%, 4% and 5%) of citric acid. Fingerlings in triplicate tanks were fed at the rate of 5% of live wet weight. Fifteen *Cirrhinus mrigala* fingerlings were stocked in V-shaped tanks with the capacity of 70 liter water twice daily, for ten weeks in feeding trial. Feces from each tank were collected twice a day and were dried at 60°C and grinded for analysis. The result showed that *Cirrhinus mrigala* fingerlings fed canola meal based diet with 3% citric acid indicated significant improvements ($p<0.05$) in weight gain (352.65%), SGR (1.68%) and minimum FCR (1.21). From the results, it was concluded that 3% citric acid supplementation in canola meal based diet is the optimum level for growth performance in *Cirrhinus mrigala* fingerlings.

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Introduction

One of the Indian major carp species, *Cirrhinus mrigala* usually well-known as "mori" is cultivated in Pakistan. *Cirrhinus mrigala* is basically a bottom feeder, feeds on plant debris and decomposing organic matter (Hussain *et al.*, 2010) which are grown with species of other major carps as well as Chinese carps and are in general, fed with diets prepared by single feed ingredient or combination of different plant by-products (Hussain *et al.*, 2011a).

Now-a-days, the highest emergent food producing industry is aquaculture industry (FAO, 2014) due to the requirement of high quality fish protein to fulfill human's nutritional requirements (Tacon and Metian, 2008; Naylor *et al.*, 2009). Fish meal is a primary protein source in aqua feed as it is a magnificent source of basic nutrients such as inevit-able amino acids, essential fatty acids, vitamins, minerals as well as a lot of growth elements (Dawood *et al.*, 2015). Moreover, it provides highly diges-tible energy as well as enormously suitable nutrients (Tacon, 1993). However, rising demand, unbalanced supply, invariable level of production and high cost of the fish meal with the development of aquaculture make compulsory to search for unconventional protein sources (Lech and Reigh, 2012; Dedeke *et al.*, 2013; Hussain *et al.*, 2014a; Hussain *et al.*, 2015b). Furthermore, its use in aqua-feeds also negatively affects the water environment by liberating P and N in water bodies (Hardy, 2002).

It was observed that oilseed meal based diets are vital source of protein and energy for aqua-feed to make environment friendly and cost-effective fish feed (Cheng and Hardy, 2002). But fish has to face several problems with the usage of oilseed meal-based diet, because there is no proper digestion and well absorption of oilseed meal-based diet at higher pH in digestive tract of fish. So, oilseed meal based diet has a deleterious effect on the digestive tract (Usmani and Jafri, 2002). One of the vital plants by- products that can be used in fish diet is canola meal based diet. According to McDonald *et al.* (1999) canola seed meal contains substantial amount of crude protein (38-40%) based on the superiority of the crop.

The utilization of these low cost plant proteins in aquafeeds can diminish feed costs if prepared exactly. A prospective substitute for fish meal is canola meal (Burel *et al.*, 2000a) as it has high amount of protein, which is of extremely digestible for fish (Cheng and Hardy, 2002). Canola comprises seed from *Brassica napus* and *B. rapa* with low quantity of glucosinolates in the meal and a low concentration of erucic acid as discussed by the Canola Council of Canada Canola (Bell, 1993). Canola cultivation is optimistic in Pakistan to fulfill the demands of edible oil of the country. The protein by-products of oil seed crops are reachable at low price in comparison with other sources at similar level of protein content.

Canola meal is essentially oil-source (40%-45%) and it becomes by-product of the seed after oil extraction either physically or by solvent extraction method, having a healthy protein by-product with protein content variable between 32% and 45% (Katiyar and chamola, 2003; Ranjan and Athithan, 2015). Canola meal has a high quality amino acid profile, which is high in methionine as well as cystine while it is low in lysine and arginine (Izadina *et al.*, 2010) but relatively higher to soybean meal (Mwachireya *et al.*, 1999). Nevertheless, the value of canola meal is usually lesser to fish meal and other oil seed meals such as soybean meals, which based on the vital amino acid profile (Sajjadi and Carter, 2004). So, in aqua feeds the inclusion of plant protein by-products are intensifying because of the constrained amount of fish meal which is obtained for the production of animal feeds (Gatlin *et al.*, 2007).

In association with the global requirement for safe human food and the production of environment friendly aquaculture products, acidifiers are natural organic acids or their salts that have attained significant attention as animal -feed additives (Elala and Ragaa, 2015). Consequently, researchers focused on organic acids and other additives in aqua-feeds are of special interest as a result of their beneficial effects in maintenance of feed (Luckstadt, 2006; Sing *et al.*, 2014; Ng and Koh 2016).

Numerous researches have been demonstrated on rabbits (Debi *et al*., 2010) and broilers (Ahmad *et al*., 2013; Ali *et al*., 2013) to evaluate the effects of organic acids in their diets. Some literatures showed improved growth performance of *Onchorynchus mykiss* (Sugiura *et al*., 1998), *Pagrus major* (Sarker *et al*., 2005) and *Labeo rohita* (Baruah *et al*., 2005) in response to organic acids. Organic acids in fish feed are valuable because it performs an important role to reduce the pH in the gut of fish (Hossain *et al*., 2007).

Among the organic acids, particularly application of citric acid (CA) has been expanding extensively for diet acidification due to its unique flavor and high buffering capacity (Shah *et al*., 2015a). Citric acid decreases the pH of digestive system (Baruah *et al*., 2005). It stimulates feed consumption that respond in better absorption of nutrients (Khajpour and Hosseini, 2012) and improves better growth performance of fish by lowering the pH in the digestive tract through cation (H^+ ion) deposition (Khajepour and Hosseini, 2012a; Castillo *et al*., 2014; Koh *et al*., 2014).

Less information is available about the *Cirrhimus mrigala* in response to organic acids. Research is required not only to explore the best and least cost plant protein sources supplemented with citric acid for commercially important species of carps such as *Cirrhinus mrigala* to enhance the growth performance but also to overcome the problems of expensive fish meal. Hence, the purpose of present study was to investigate the effects of citric acid supplementation on growth performance of *Cirrhinus mrigala* fingerlings fed canola meal based diet and to improve production of this indigenous culture able fish.

Material and methods

The present research work was carried out to investigate the effects of citric acid supplementation on growth parameters of *Cirrhinus mrigala* fingerlings fed on Canola meal based diet. The experiment was conducted in the Fish Nutrition Laboratory, Department of Zoology Government College University, Faisalabad.

Fish acclimatization and experimental conditions

Cirrhinus mrigala fingerlings were bought from Government Fish Seed Hatchery, Satiana road Faisalabad and were acclimatized to experimental conditions in laboratory for two weeks in V-shaped tanks (GCUF system) having capacity of 70 L water specifically designed for the collection of fecal material from water media.

Fifteen fish were stocked in each tank. *Cirrhinus mrigala* fingerlings were treated before start of the feeding trial with NaCl (5g L^{-1}) to free from ectoparasites and also to prevent further fungal infection (Rowland and Ingram, 1991). 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). To maintain the level of dissolved oxygen, air was provided 24 hours by using air pump.

Feed ingredients and experimental diets

The feed ingredients were brought from a commercial feed mill and were analyzed for chemical composition by following standard methods (AOAC, 1995) prior to the formulation of the experimental diet. The feed ingredients were grinded and sieved to pass through 0.5 mm sieve size before incorporation with experimental diet (Table 1).

All dry ingredients were mixed in electric blender for 10-20 minutes, thereafter fish oil was gradually added, while mixing continually. Feed were divided into 6 groups, each having 1kg weight.

Citric acid was mixed in six canola meal based diets at the levels of 0%, 1%, 2%, 3%, 4% and 5%. Chromic oxide (1%) was added as an inert marker. Ten to fifteen percent water was slowly included to prepare suitable dough of each test diet, and was further processed through pelleting machine for making pellets (Lovell, 1989).

Table 1. Ingredient composition (%) of control and canola seed meal based diets.

Ingredients	Test diet-I	Test diet-II	Test diet-III	Test diet-IV	Test diet-V	Test diet-VI
Canola meal	52%	52%	52%	52%	52%	52%
Citric Acid	0%	1%	2%	3%	4%	5%
Fish Meal	13.0%	13.0%	13.0%	13.0%	13.0%	13.0%
Wheat Flour*	12 %	12 %	12 %	12 %	12 %	12 %
Rice Polish	13%	13%	13%	13%	13%	13%
Fish Oil	6%	6%	6%	6%	6%	6%
Vitamin Premix	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%
Minerals Premix	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%
Ascorbic Acid	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%
Chromic Oxide	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%
Total	100%	100%	100%	100%	100%	100%

*Citric acid was added at the expense of wheat flour.

Feeding Protocol

The fingerlings of *Cirrhinus mrigala* were fed at the rate of 5% of live wet weight on their recommended diets twice daily (morning and afternoon). For each test diet, triplicate tanks were used with stocking density of fifteen fish in each. After the feeding session of two hours, the uneaten diet was drained out from each tank by opening the valves of the tanks. The tanks were washed completely to remove the particles of diets and refilled with water. After that, the fish was restocked in tanks. The experiment was lasted ten weeks for the collection of 5 g fecal material of each replicate. During the study period, Water quality variables, particularly temperature (20-30°C), pH (6.7-7.7) and dissolved oxygen (8.68 mg/L-10.92mg/L) were monitored by the usage of Jenway pH meter model 3510 and D.O. meter model 970 respectively. Aeration was provided round-the-clock to all the tanks through capillary system.

Growth study

Fish in each tank were bulk weighed every 15th day during research work to evaluate the growth performance of *Cirrhinus mrigala* fingerlings. Wight gain (%), Feed conversion ratio and Specific growth rate SGR (%) was assessed with standard formulae.

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

$$\text{FCR} = \frac{\text{Total dry feed intake (g)}}{\text{Wet weight gain (g)}} \times 100$$

SGR% = 100 {ln. final wt. of fish – ln. initial wt. of fish} / trial day.

Statistical analysis

Finally, data of growth parameters of fish were subjected to one-way analysis of variance (Steel *et al.*, 1996). The differences among treatments were compared by Tukey's Honesty Significant Difference Test and considered significant at $P<0.05$ (Snedecor and Cochran 1991). The Co Stat Computer Package (version 6.303, PMB 320, Monterey, CA, 93940 USA) was used for statistical analysis.

Results

The results of growth efficiency of *Cirrhinus mrigala* fingerlings fed on canola meal based test diets supplemented with graded levels of citric acid are presented in tables (3 and 4). It was observed that canola meal based diet supplemented with CA enhanced the growth performance of fish. Weight gain started increasing from the test diet II at the 1% level of citric acid and continued to the 3% level. A significant ($P<0.05$) increase in weight gain was noted with 3% level of citric acid after which further increase in citric acid supplementation steadily decreased the weight gain of fish. The maximum value of weight gain (23.33±0.15 g) of *Cirrhinus mrigala* fingerlings was observed on canola meal based test diet having 3% level of citric acid and this value was significantly different ($P<0.05$) from the weight gain on control diet and other citric acid supplementation based test diets. The significant increase (352.65±0.92%) in weight gain (%) was also observed at 3% level and this was more than fish fed control diet (218.47±5.65%). The minimum weight gain (231.16±4.21g) was recorded at 5% level of citric acid supplementation.

The results exhibited that weight gain on control diet and test diet VI containing 5% level of citric acid, were statistically similar. Results indicated that citric acid supplementation at 3% level is the optimum level for maximum weight gain as in comparison with control diet.

It was further observed that 0%, 1%, 2%, 4% and 5% levels of citric acid supplementation could not demonstrate significant improvement in weight gain of *Cirrhinus mrigala* fingerlings as compared 3% citric acid supplemented diet.

Table 2. Chemical analysis (%) of feed ingredients (Dry matter basis)

Ingredients	Dry matter (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	Ash (%)	Gross Energy (kcal/g)	Carbohydrates (%)
Fish meal	991.53	446.17	76.15	11.13	24.23	4.07	18.25
Wheat flour	992.53	110.54	22.36	22.59	2.81	2.86	78.84
Rice polish	994.78	112.56	112.75	111.54	10.89	4.36	47.81
Canola meal	993.52	337.10	1.35	11.39	8.27	3.15	48.74

Table 3. Weight gain and weight gain (%) of *Cirrhinus mrigala* fingerlings fed citric acid supplemented canola meal based test diets.

Experimental Diets	Citric acid levels (%)	Initial Weight (g)	Final Weight (g)	Weight Gain (g)	Weight Gain (%)
Test Diet –I (Control)	0	6.62 ± 0.08^{a}	21.08 ± 0.17^{f}	14.46 ± 0.23^{f}	218.47 ± 5.65^{e}
Test Diet –II	1	6.57 ± 0.05^{a}	23.09 ± 0.62^{d}	16.53 ± 0.57^{d}	251.65 ± 7.11^{d}
Test Diet –III	2	6.67 ± 0.04^{a}	25.10 ± 0.20^{c}	18.43 ± 0.24^{c}	276.66 ± 5.29^{c}
Test Diet –IV	3	6.62 ± 0.05^{a}	29.95 ± 0.20^{a}	23.33 ± 0.15^{a}	352.65 ± 0.92^{a}
Test Diet –V	4	6.61 ± 0.05^{a}	27.08 ± 0.10^{b}	20.47 ± 0.15^{b}	309.55 ± 4.55^{b}
Test Diet –VI	5	6.63 ± 0.06^{a}	21.94 ± 0.10^{e}	15.32 ± 0.15^{e}	231.16 ± 4.21^{e}

Means within rows having different superscript are significantly different at $p<0.05$. Data are means of three replicates.

Table 4. FCR and SGR% of *C. mrigala* fingerlings fed citric acid supplemented canola meal based diets

Experimental Diets	Citric acid levels (%)	Weight Gain $fish^{-1} day^{-1}$ (g)	Feed intake $fish^{-1} day^{-1}$ (g)	FCR	SGR (%)
Test Diet –I (Control)	0	0.21 ± 0.00^{f}	0.35 ± 0.01^{c}	1.68 ± 0.00^{a}	1.29 ± 0.02^{f}
Test Diet –II	1	0.24 ± 0.01^{d}	0.36 ± 0.01^{c}	1.53 ± 0.00^{c}	1.40 ± 0.02^{d}
Test Diet –III	2	0.26 ± 0.00^{c}	0.38 ± 0.01^{b}	1.44 ± 0.01^{d}	1.47 ± 0.01^{c}
Test Diet –IV	3	0.33 ± 0.00^{a}	0.40 ± 0.00^{a}	1.21 ± 0.01^{f}	1.68 ± 0.00^{a}
Test Diet –V	4	0.29 ± 0.00^{b}	0.39 ± 0.00^{ab}	1.32 ± 0.00^{e}	1.57 ± 0.01^{b}
Test Diet –VI	5	0.22 ± 0.00^{e}	0.34 ± 0.00^{c}	1.57 ± 0.01^{b}	1.33 ± 0.01^{e}

Means within rows having different superscript are significantly different at $p<0.05$. Data are means of three replicates.

Maximum feed intake was also observed at 3% level of citric acid while the minimum feed intake was noted on 5% level of citric acid as in comparison with control diet. Feed intake on other levels based diets was slightly different from each other. Table 4 is depicting that the FCR of *Cirrhinus mrigala* fingerlings was also significantly ($P<0.05$) improved by adding citric acid. The best value of FCR was observed (1.21±0.01) on test diet IV having 3% level of citric acid supplementation as compared to FCR of fish fed control diet.

The decreased FCR value showed that maximum feed taken by fish was converted into flesh. FCR value started increasing with the increasing level of citric acid supplementation after 3% level. The poor value of FCR was shown by fish fed on canola meal based diet supplemented by 0% and 5% level of citric acid showing that at these levels minimum feed was converted into flesh. Similarly, results of SGR (%) also showed that the 3% level of citric acid enhanced the specific growth rate (1.68) of fish.

Minimum values of SGR (%) were observed in fish fed at the 0% and 5% level of citric acid supplemented test diets, respectively.

Discussion

Reda *et al.* (2016) observed that dietary acidifiers have favorable effects in aqua cultural production due to their better feed utilization and growth performance. Study of growth is an important parameter of fish culture competence. Minute detail is known about acidifier use in one of the major carps (*C. mrigala* fingerlings). In the current study, *C. mrigala* fingerlings were fed a canola meal based diet supplemented with citric acid at graded (1%, 2%, 3%, 4% and 5%) levels and compared with fish fed having a control diet (0% level of citric acid). The findings of present study showed that CA at the level of 3% is optimum level for growth parameters. Although research work was carried out throughout the world on feed supplemented with organic acid yet data related to growth performance on *Cirrhinus mrigala* and other carps is quite few and far between. Mostly researchers focus on the weight gain % values to determine the energetic effects of food supplements but in current research a wide range of parameters are selected to know growth parameters of *Cirrhinus mrigala* fingerlings. FCR, SGR % and weight gain % values of current research indicated that fingerlings fed on diet IV containing 3% supplemental level of CA was in good condition with FCR value (1.21±0.01), SGR % value (1.68±0.00) and weight gain % value (352.65±0.92). The addition of 3 % CA in the diets increased the WG%, SGR% and reduced the FCR value in the present study, which is in concurrence with other indications and investigated in red sea bream (Sarker *et al.*, 2005), rohu (Baruah *et al.,* 2007b), beluga (Khajepour and Hosseini, 2012a) and common carp (Khajepour *et al.,* 2012). Many researchers found different graded levels of citric acid to be affective as far as growth performance of fish fingerlings is concerned. In contrast of our findings, several other studies, in red sea bream *P. major* (Hossain *et al.,* 2007; Sarker *et al.,* 2007), in rainbow trout *O. mykiss* (Panday and Satoch, 2008; Tabrizi *et al.*, 2012) and in Yellowtail (Sarker *et al.,* 2012b) 1% level of CA was optimum for maximum weight gain (%) and FCR. This inconsistency seen in the results of different researchers may be related to unpredictable factors such as water quality parameters, feed ingredients and citric acid sources (Liu *et al.*, 2013; Olusola and Nwanna, 2014).

In conclusion, the current study provided evidence that citric acid supplemented in canola meal based diets increased growth parameters of *Cirrhinus mrigala* fingerlings. From the current research work, it is confirmed that citric acid can be supplemented in fish feeds based on plant protein source to enhance fish growth performance and play a significant role in developing environment friendly and economical feed for indigenous culturable major carp i.e. *C. mrigala*. It is recommended that further studies should be conducted to investigate the more appropriate level of citric acid supplementation near 2% and 3% levels under different dietary and rearing conditions.

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