



Full Length Article

Effect of Citric Acid Acidified *Moringa oleifera* Seed Meal Based Diet on Nutrients Digestibility and Growth Performance of *Cirrhinus mrigala* Fingerlings

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Abstract

This research work was conducted to evaluate the effect of citric acid (CA) acidified *Moringa oleifera* (Lamarck) seed meal (MOSM) based diet on the apparent digestibility coefficients and growth performance of *Cirrhinus mrigala* (Hamilton, 1822) fingerlings. MOSM based diet was sub-divided into one control diet (0% CA) and five test diets, each supplemented with 1, 2, 3, 4 and 5% CA, respectively. Ten fingerlings of *C. mrigala* were stocked in V-shaped water tanks used in triplicate for each treatment. The feed was given at 5% live wet body weight of fingerlings for 90 days. Results showed that 3% CA acidified diet significantly enhanced ($p < 0.05$) the apparent digestibility of crude protein (67.93%), crude fat (71.43%) and gross energy (69.00 kcal/g). Diet acidification with CA also significantly ($p < 0.05$) improved overall growth performance of *C. mrigala* fingerlings as compared to control diet. The acidification of MOSM based diet with 3% CA significantly ($p < 0.05$) increased weight gain (WG; 24.45 g), weight gain percent (WG; 255.73%), specific growth rate (SGR; 1.41) and significantly ($p < 0.05$) decreased feed conversion ratio (FCR; 1.29). In conclusion, 3% CA acidified MOSM based diet is recommended for better nutrients digestibility and growth performance of *C. mrigala* fingerlings. © 2017 Friends Science Publishers

Keywords: Citric acid; Moringa; Nutrient digestibility; Growth; Fingerlings

Introduction

Production of freshwater fishes has always been dominated by carps (FAO, 2012). Carps are cultured throughout the world in animal aquaculture and well recognized for their adaptability to grow rapidly in confined waters. These fishes are herbivorous and are very selective about their food in nature (Desilva, 2003; Singh *et al.*, 2006). *Cirrhinus mrigala* is one of major Indian carps commonly consumed throughout Pakistan because of its good meat quality and taste. It has wide distribution in freshwater reservoirs of Pakistan of substantial economic importance and market value (FAO, 2007; Rauf, 2015).

Supplementary feed constitutes more than 50% expenditure in carp. Therefore, proper feed management is fundamental for enhancing profits (FAO, 2006). Formulation of balanced diets for the successful intensive culture of carps depends upon the knowledge of specific protein requirements for these species. Fishmeal and fish oil are commonly used throughout the world as carp feed, although they are not essential on the purely nutritional basis (Bostock *et al.*, 2010). The furthermore rapid increase

in fish oil and fishmeal prices can have negative affect the commercial aquaculture projects (Naylor *et al.*, 2000). Therefore, it is recommended to include plant protein sources in carp diet because of their low cost, abundant availability and herbivorous nature of fishes (Baruah *et al.*, 2007). It is of utmost importance that plant-based ingredients used in aquaculture feed contain certain nutritional characteristics, such as low levels of starch, fiber, anti-nutrients and relatively higher protein contents, high nutrient digestibility, favorable amino acid profile and reasonable palatability (Naylor *et al.*, 2009).

Moringa oleifera is one of the potential plant protein sources for inclusion in aquaculture diets (Chiseva, 2006). The plant of Moringa is high yielding with rapid growth rate. Pods begin to appear after 6 to 8 months of planting but regular fruiting starts after two years and continue for 30 to 40 years (Chuks *et al.*, 2013). Moringa kernel and the fat-free kernel meals have 36.7% and 61.4% of crude protein, respectively. It is also potential protein substitute in fishmeal based diets which can minimize the use fishmeal in aquaculture due to its nutritional value of total soluble protein and high pepsin. Because of the favorable amino

acid profile and wide availability throughout the tropical and subtropical regions, Moringa leaves and seeds can be regarded as a potential feed component of fish diet to make aquaculture production cost effective (Tagwireyi *et al.*, 2014).

Digestibility of feed nutrients is a key factor in aquaculture production efficiency and also impacts the environment (NRC, 2011). There are certain factors such as phytate which limits the bioavailability of nutrients present in plant-based fish diets (Francis *et al.*, 2001). Dietary acidification is recommended in fish nutrition to release bound nutrients in phytate (Jongbloed, 1987) because acid secretion in the fish gut is always lower compared to mammals. Lower pH in fish gut kills the pathogens, increases hydrolysis of phytate, decreases gastric emptying and improves nutrients absorption (Shah *et al.*, 2015). Among the organic acids, CA has been widely used for dietary acidification because of its high buffering capacity and unique flavor (Hossain *et al.*, 2007).

C. mrigala is a fish of great economic importance along with two other Indian major carps such as *Labeo rohita* and *Catla catla*. It contributes a major share to the freshwater aquaculture production throughout the Indian subcontinent (Biju *et al.*, 2003). Economic efficiency of commercial aquaculture depends upon the growth rate of fish. Studies performed to explore the growth and feed performance responses to CA acidified diets have shown encouraging results (Sarker *et al.*, 2005; Pandey and Satoh, 2008). Nutrients digestibility and potential for nutrient retention and waste production are factors should be considered for sustainable animal production when considering diet formulations (Hixson, 2014). It was hypothesized that CA supplementation in MOSM based diet improves the growth and nutrient digestibility of *C. mrigala* fingerlings. Therefore, the present study was conducted to investigate the effect of CA acidified MOSM based diets on nutrient digestibility and growth performance of *C. mrigala* fingerlings.

Materials and Methods

Fish and Experimental Conditions

Experimental fish Mori (*Cirrhinus mrigala*) fingerlings were obtained from "Government Fish Seed Hatchery", Faisalabad. Before starting the feeding trial, fingerlings were dipped in NaCl (5 g/L) solution for specific time to make them free of ectoparasites. The fingerlings were acclimatized in the laboratory for 2 weeks period in V-shape like water tanks particularly designed to collect fish fecal material. Throughout this period fingerlings were fed once daily on a basal diet to apparent satiation (Allan and Rowland, 1992). Physical parameters like temperature, dissolved oxygen, and pH were monitored on a daily basis. Tap water was used during the whole experiment.

Ingredients of Feed and Experimental Diets Formation

The ingredients of feed were procured from a commercial feed mill and their chemical composition was analyzed (AOAC, 1995) before the formulation of the test diets (Table 1). The ingredients of feed were ground finely to pass through (0.5 mm) mesh size. All Ingredients of feed were mixed up for five minutes in a food-mixer and fish oil was added subsequently. MOSM based diet was sub-divided into one control (0%) 0 g CA and five test diets, each having 5 kg weights supplemented with 1% (50 g), 2% (100 g), 3% (150 g), 4% (200 g) and 5% (250 g) CA, respectively. The 100 g feed per 85 mL of water was blended into the food-mixer to form a suitable dough, and the pelleting machine was used to process this dough into pellets (Lovell, 1989).

Feeding Schedule and Collection of Sample

C. mrigala fingerlings were fed twice daily (8:00 am and 2:00 pm) on their respective diet at the rate of 5% live wet body weight. Each test diet was used in three replicate and fifteen fingerlings were used in each replicate. After two hours feeding session, the unutilized diet from each tank was collected through the valves of tanks. The tanks were washed completely to remove diet particles and water was refilled in each tank. 3 h after the feeding session, feces were collected from each tank through fecal collection pipes using valves. Utmost care was done to avoid breakdown of fecal strings during fecal collection to make sure minimized nutrient leaching. Fecal material from each tank was oven dried at 60°C, completely ground and stored in the lab for chemical analysis. A 5 g fecal material was collected from each tank until the completion of 90 days feeding period.

Chemical Analysis of Feed and Feces

The samples of feces, experimental diets and feed ingredients were homogenized separately by motor and pestle and were analyzed by standard procedures (AOAC, 1995). Crude protein ($N \times 6.25$) was determined by micro Kjeldahl apparatus; moisture by 12 hours oven-drying at 105°C; crude fat using Soxtec HT2 1045 system through petroleum ether extraction method and gross energy by oxygen bomb calorimeter.

Estimation of Chromic Oxide

Chromic oxide as an inert marker was added in test diets to determine nutrient digestibility. Chromic oxide content after oxidation of the ash samples of feces and experimental diets with perchloric reagent was estimated by acid digestion method (Divakaran *et al.*, 2002) through UV-VIS 2001 spectrophotometer at 350 nm.

Table 1: Ingredients composition (%) of test diets

Ingredients	Test Diet-I (control)	Test Diet-II	Test Diet-III	Test Diet-IV	Test Diet-V	Test Diet-VI
MOSM	35	35	35	35	35	35
Fish meal	15	15	15	15	15	15
Soybean meal	15	15	15	15	15	15
Wheat flour	17	17	17	17	17	17
Rice polish	8	8	8	8	8	8
Fish oil	6	6	6	6	6	6
Vitamin Premix	1.0	1.0	1.0	1.0	1.0	1.0
Mineral 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
Citric acid level	0 %	1 %	2 %	3 %	4 %	5 %
Total	100.0	100.0	100.0	100.0	100.0	100

MOSM=Moringa seed meal; Citric acid will be used at the expense of wheat flour

Digestibility Calculation

ADC% (Apparent nutrient digestibility coefficients) for test diets was determined using standard formula (NRC, 1993).

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

Growth Studies

Fingerlings in each replicate were bulk weighed after the completion of experiment to determine the growth. Fish growth performance was determined using standard formulae:

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

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

Statistical Analysis

The nutrient digestibility and growth performance data were analyzed by one-way analysis of variance (Steel *et al.*, 1996). Tukey's Honesty Significant Difference Test was used to compare differences among treatments and considered significant at $p < 0.05$ (Snedecor and Cochran, 1991). Statistical analysis was done using Co-Stat Computer Package (version 6.303, PMB 320, Monterey, CA, 93940 USA).

Results

Analyzed composition of nutrients in MOSM based diet and feces of *C. mrigala* fingerlings are represented in Table 2 and Table 3. Apparent crude protein, crude fat and gross energy digestibility of *C. mrigala* fingerlings fed MOSM based diets is represented in Table 4. Fingerling fed CA acidified diets showed significantly ($p < 0.05$) improved digestibility of nutrients compared to control diet. Maximum crude protein digestibility (67.93%) was observed at 3% CA level followed by 66.45% crude protein digestibility at 4% CA level. However, minimum crude protein digestibility (48.59%) was observed in control diet. Whereas, maximum crude fat digestibility (72.43%) was

observed in fingerlings fed at 2% CA acidified MOSM based diet. Fingerling fed control diet and 5% CA acidified MOSM based diet showed significantly ($p > 0.05$) lower crude fat digestibility compared to all other CA acidification levels. Significantly ($p < 0.05$) higher gross energy digestibility (69.00 kcal/g) was observed in fingerlings fed 3% CA acidified MOSM based diet. Fingerlings fed 2 and 4% CA acidified MOSM based diets showed significantly better gross energy digestibility compared to control diet and 1 and 5% CA acidified MOSM based diets. Fig. 1 shows the relationship between CA acidified diets and nutrient digestibility parameters. The value of R^2 for crude protein (0.860), crude fat (0.965) and gross energy (0.884) digestibility reveals that more than 85% variation in above-said parameters is explained by CA. Optimum crude protein digestibility (66.39%), crude fat digestibility (71.48%) and gross energy digestibility (67.40%) were revealed at optimal CA levels 4.63, 2.53 and 2.89%, respectively (Fig. 1).

Growth performance of *C. mrigala* fingerlings fed CA acidified MOSM based diets are presented in Table 5. The results showed that acidification of MOSM based diet with CA significantly improved overall growth performance of *C. mrigala* fingerlings as compare to control diet. Dietary acidification at 3% CA level significantly improved WG (24.45 g), WG% (255.73%) and SGR (1.41) as compare to other CA levels. FCR value significantly improved at 2, 3 and 4% CA level as compare to control, 1 and 5% CA levels. The relationship between CA and various growth parameters is presented in Fig. 2. Estimated curve showed change in various growth performance parameters with change in CA in MOSM based diets. R^2 value for WG (0.916), WG% (0.919), FCR (0.991) and SGR (0.927) revealed that more than 90% variation in above said parameters is caused by CA.

Discussion

M. oleifera tree is of great economic importance with many uses and found throughout most of the tropical regions (Abbas, 2013). Because of the favorable amino acid profile and wide availability throughout the tropical and subtropical regions (Tagwireyi *et al.*, 2014),

Table 2: Analyzed composition of nutrients in MOSM based test diets

Diets		Crude protein (%)	Crude fat (%)	Gross energy (kcal/g)
Citric acid Levels (%)	0 (Control diet) (Test diet I)	31.37	7.56	3.24
	1 (Test diet II)	31.36	7.56	3.25
	2 (Test diet III)	31.36	7.57	3.25
	3 (Test diet IV)	31.36	7.58	3.25
	4 (Test diet V)	31.37	7.56	3.26
	5 (Test diet VI)	31.35	7.57	3.26
PSE		0.06284903	0.07929081	0.0603846
<i>p</i>		0.9999	0.0061267	0.9999

Data are means of three replicates; PSE =pooled SE = $\sqrt{\text{MSE}/n}$ (where MSE=mean-squared error)

Table 3: Analyzed nutrients (%) in feces of *C. mrigala* fingerlings fed MOSM based diets

Diets		Crude protein (%)	Crude fat (%)	Gross energy (kcal/g)
Citric acid Levels (%)	0 (Control diet) (Test diet I)	17.44a	3.65a	1.98a
	1 (Test diet II)	14.82b	3.00b	1.80ab
	2 (Test diet III)	14.96b	2.24c	1.18c
	3 (Test diet IV)	10.71d	2.31c	1.07c
	4 (Test diet V)	11.01cd	2.81b	1.18c
	5 (Test diet VI)	11.81c	3.62a	1.74b
PSE		0.18052396	0.04727297	0.04386646
<i>p</i>		0.0000	0.0000	0.0000

Data are means of three replicates; PSE =pooled SE = $\sqrt{\text{MSE}/n}$ (where MSE=mean-squared error)

Table 4: Apparent nutrients digestibility (%) of *C. mrigala* fingerlings fed MOSM based diets

Diets		Crude protein (%)	Crude fat (%)	Gross energy (kcal/g)
Citric acid Levels (%)	0 (Control diet) (Test diet I)	48.59d	55.34c	43.51d
	1 (Test diet II)	56.60c	63.51b	49.15c
	2 (Test diet III)	55.63c	72.43a	66.19b
	3 (Test diet IV)	67.93a	71.43a	69.00a
	4 (Test diet V)	66.45ab	64.41b	65.45b
	5 (Test diet VI)	65.40b	56.13c	51.01c
PSE		0.45055606	0.4932203	0.48159793
<i>p</i>		0.0000	0.0000	0.0000

Data are means of three replicates; PSE =pooled SE = $\sqrt{\text{MSE}/n}$ (where MSE=mean-squared error)

Table 5: Growth performance of *C. mrigala* fingerlings fed MOSM based test diets

Experimental diets	Citric acid (%)	Initial weight (g)	Final weight (g)	Weight gain (g)	Weight gain (%) (Fish-1 day-1) g	Feed intake	FCR	SGR	
Test diet-I (Control diet)	0	9.57	28.76f	19.19f	200.60e	0.21f	0.34ab	1.61c	1.22e
Test diet-II	1	9.55	30.33d	20.78d	217.65c	0.23d	0.33c	1.44b	1.28c
Test diet-III	2	9.56	33.01b	23.45b	245.33b	0.26b	0.34bc	1.30a	1.38b
Test diet-IV	3	9.56	34.02a	24.45a	255.73a	0.27a	0.35a	1.29a	1.41a
Test diet-V	4	9.54	32.54c	23.00c	241.09b	0.26c	0.34bc	1.31a	1.36b
Test diet-VI	5	9.54	29.35e	19.81e	207.59d	0.22e	0.32d	1.45b	1.25d
PSE		0.046047	0.08425	0.095539	1.705658	0.001062	0.005286	0.021926	0.006242
<i>p</i>		0.9924	0.0000	0.0000	0.0000	0.0000	0.5848	0.0000	0.0000

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

PSE =pooled SE = $\sqrt{\text{MSE}/n}$ (where MSE=mean-squared error)

Moringa seeds were used as protein source for the formulation of MOSM based diets. Acidification of diet with CA release bound nutrients in the diet and also increase its acceptability to fish (Baruah, 2004). Therefore, MOSM based diets were acidified with CA to check their effect on nutrients digestibility and growth performance of *C. mrigala* fingerlings.

The present study revealed that CA acidified MOSM based diets significantly improved overall nutrients digestibility of *C. mrigala* fingerlings. Positive results of CA

acidified diets on digestibility of nutrients are also reported by (Baruah *et al.*, 2007; Hussain *et al.*, 2015; Rabia *et al.*, 2017). This may be because CA lowers intestinal pH to increase the efficiency of digestive enzymes thus improving the digestibility of nutrients. In present study fingerlings fed 3% CA acidified diet showed significantly higher nutrients digestibility. In agreement with our findings Baruah *et al.* (2007) reported in his study that 3% CA acidified and phytase treated diet synergistically enhanced the nutrient digestibility of *Labeo rohita* juveniles.

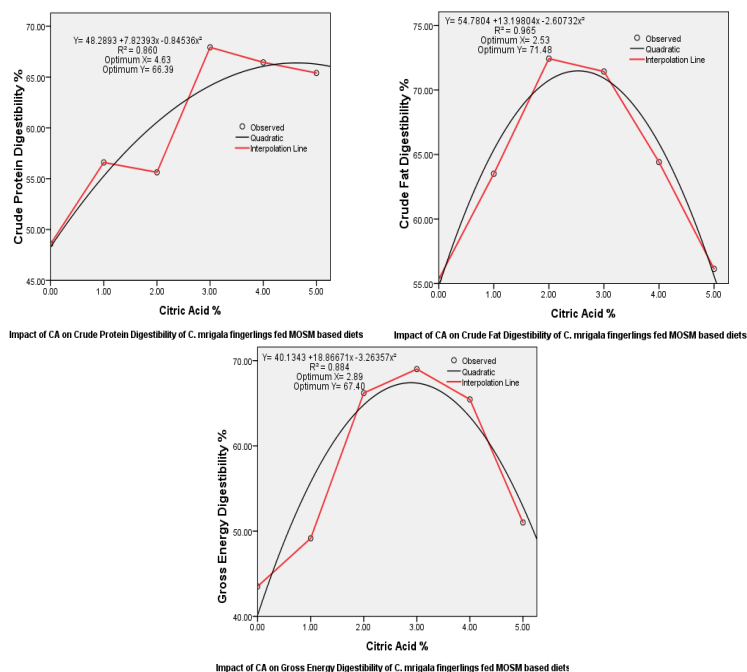


Fig. 1: Relationship between CA and various nutrient digestibility parameters of Indian major carps fingerlings fed MOSM based diets

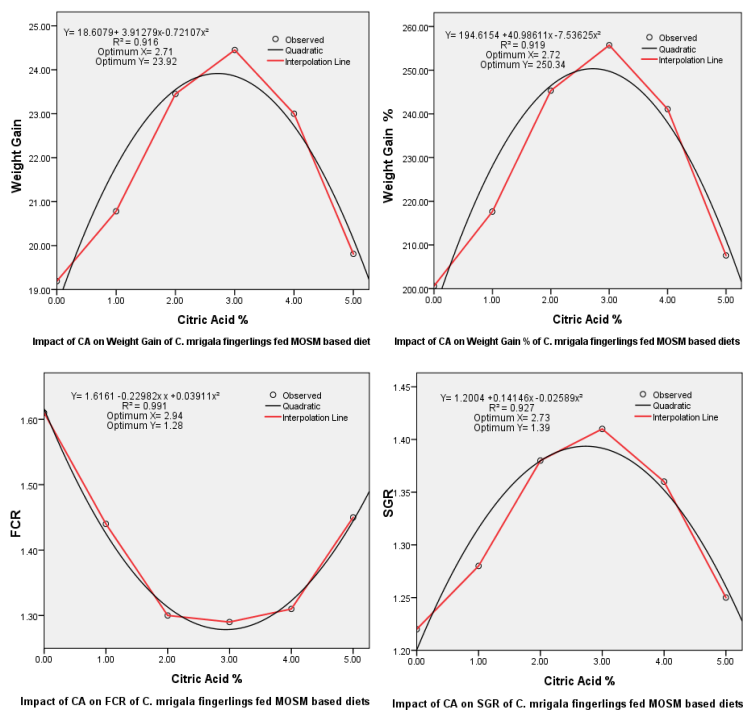


Fig. 2: Relationship between CA and various growth performance parameters of *C. mrigala* fingerlings fed MOSM based diets

These results disagree with findings of Rabia *et al.* (2017) and Afzal *et al.* (2016) who concluded that dietary inclusion of 2% citric acid in sunflower meal-based diet improved the nutrient digestibility in *L. rohita* juveniles. Further disagreeing with us Hussain *et al.* (2015) reported the

significantly higher dry matter, crude protein, crude fat and gross energy digestibility in *C. mrigala* fingerlings fed 5% CA acidified corn gluten meal based diet. This disagreement in results may be because they used different diets and levels of CA in their experiments.

In the present study CA acidified diets improved overall growth performance of *C. mrigala* fingerlings as compared to control diet. Improved growth response to CA acidified diet is also reported by Baruah et al. (2007), Afzal et al. and Shah et al. (2016) in *Labeo rohita*; Hussain et al. (2015) in *C. mrigala*. This improved growth performance in fish fed CA acidified diet is may be because CA increases the phytate dephosphorylation to release of bound nutrients thus improving growth performance of fish (Baruah et al., 2007). The present study revealed that fingerlings fed 3% CA acidified diet showed significantly improved WG, WG%, SGR and FCR. Our results are in agreement with Baruah et al. (2007) and Asrar et al. (2016) who also reported improved WG, SGR and FCR value in *L. rohita* and *C. mrigala* fed 3% CA acidified diet, respectively. Sarker et al. (2005) and Shah et al. (2016) also reported improved growth parameters of fish fed 3% CA acidified diets. In contrary to our results Afzal et al. (2016) and Hussain et al. (2015) reported better growth performance of fish fed 2 and 5% CA acidified diets.

Conclusion

Addition of CA in plants based diets release bound nutrients and significantly improves nutrients digestibility and growth performance of fish. Acidification of MOSM based diet with 3% CA is recommended for better nutrients digestibility and growth performance of *C. mrigala* fingerlings.

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