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Research Article

Acidified Diet Increases the Trace Mineral Content in Whole Body Fish *Labeo rohita* Fingerlings

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Authors' Contributions
LS executed the research. MA supervised the study. SZHS did statistical analysis. MF helped in compiling the data. HN and QS helped in writing the article.

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Fish, Minerals, Citric acid, Diet, Supplementation.

Abstract | The present study was designed to check minerals in fish body with organic acid supplemented diets in *Labeo rohita* fingerlings. Five experimental diets were prepared containing organic acid (0%), malic acid (2%), citric acid (2%), formic acid (2%), lactic acid (2%) and designed as "OA_1, OA_2, OA_3, OA_4 and OA_5", respectively. Eight weeks experimental trail were performed. Water quality characteristics (dissolved oxygen, pH and temperature) were checked daily throughout the trail. Results concluded that the body mineralization in *L. rohita* was increased by supplementation of organic acid. The maximum body mineralization was noted in fish fed with the dietary group OA_3 that contain citric acid and minimum mineralization was noted in the diet OA_5 containing lactic acid when compared with other dietary treatments. In conclusion, citric acid showed best response as compared to other dietary supplemented organic groups.

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Introduction

The little use of antibiotics in animal feed proves beneficial because it gave immunity against bacterial pathogens (Liem, 2004). The residual antibiotics, which are present in Sea food products that caused health problem in consumers, if these antibiotics added in fish feed (Nawaz *et al*., 2011). For these issues, Europeans union has been banned the growth promoter antibiotics in animal feed. As a result, alternatives of antibiotic growth promoters are required for sustainable aquaculture in all over the world (Parks *et al*., 2001). Many studies (Parks *et al*., 2001) proves that the use of organic acid and probiotics in diet showed positive results in animal performance.

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Fish meal is an important component of commercial feed particularly used in rearing of carnivorous species, such as trout, marine species and salmon. Now days, fish meal based diet has been replaced with plant based products due to very limited resources of fish meal. In comparison of fish meal, soybean meal is an excellent source of protein which has high protein and low phosphorous content and is easily available (Hardy, 1995). Therefore, soybean meal has two-third of phosphorous in the form of phytate which is not easily consumed by fish (NRC, 1993). Phytate is hydrolysed by organic acid. Citric acid in fish feed enhanced the solubility of calcium and phosphorous and also improve the utilization of minerals (Vielma *et al*., 1999). Supplementation of formic acid in the semi moist diet which has fish protein and fishmeal enhanced the accessibility of calcium, phosphorous and magnesium in rain bow trout (Vielma *et al*., 1998). However, lactic acid in fish diet decreases the intestinal pH and act as chelating agent which binds many cations with intestine (Ravindran and Kornegay, 1993).

Malic acid enhanced the digestibility of many minerals, which present in fish meal that contain phosphorous. The diet supplemented with organic acid showed maximum absorption of phosphorous. Phosphorus excretion in malic acid enhanced the phosphorous utilization (De Wet, 2005). Therefore, current work was designed to evaluate the minerals in fish, *Labeo rohita* body fed with organic acid supplemented diets.

Materials and Methods

The present research work was carried out in the Fish Nutrition Laboratory, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad. *Labeo rohita* juveniles were brought from the Fish Seed Hatchery, Faisalabad, and were acclimatized to experimental conditions for two weeks. Cemented tanks were utilized to acclimatize the *L. rohita* juveniles. Before the experiment, fish were washed with $5gL^{-1}$ NaCl to prevent from contamination of Ecto-parasite. Fish were transferred to V-shaped tanks (UA system) for experimental trail. The whole experiment was performed with two replicates for each experiment of diet. Feed was given to fish once daily during the trail. Juveniles were fed on basal diet to evident satiation (Allan and Rowland, 1992). Water quality characteristics such as temperature (30°C), pH (7.5) and dissolved oxygen (225 mgL^{-1}) were checked by using thermometer, pH meter (Jenway, modal 3510) and D.O. meter (Jenway, modal 270), respectively. Capillary system was utilized for the retention of tank round the clock. The trail was run for two months.

Feed ingredients and experimental diets

Prior to diet formulation, ingredients were brought from local poultry feed market and analyzed for chemical composition following the method of AOAC (1995). The feed ingredients were ground into optimum particle size, before being incorporated into experimental size.

Feeding protocol

Prescribed diets were given to the juveniles of *L. rohita* at the rate of 2% of live wet body weight. After three hours of sustaining session the uneaten diet was drained out of every tank by opening the valves. After every feeding session the tanks were washed, refilled with water and fishes were restocked.

Samples collection

Toward the completion of trail, clove oil solution (3000 mgL^{-1} for 40-60s) was used to anesthetize the fishes and a sharp blow was given on the head to examine them. After washing fish body, the samples were ground, minced and homogenized in sampler homogenizer.

Chemical analysis of diet

Pastle and motor were used to crush the diet samples. In order to determine moisture, samples were oven-dried at 105°C for 12 h. Crude protein was estimated by Micro kjeldhal apparatus. Soxtec HT2 1045 system were used for ether extraction. The determination of crude fiber was conducted by measuring the loss of dried lipid-free residues on ignition, after digestion with 1.25% H_2SO_4 and 1.25% NAOH. Samples were ignited in electric furnace (Eyela-TMF 3100) at 650°C for 12 h for the estimation of crude ash.

Mineral estimation of diet and fish body

Four mineral estimation, boiling nitric acid and per chloric acid mixture (3:1) was used for the digestion of diet and fish body samples (AOAC, 1995). After required dilution, using Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) was used to estimate minerals, including Zn, Cu, Fe, and Mn.

Statistical analyses

Finally, data of entire body minerals were analyzed through ANOVA following Steel *et al.* (1996). The differences among means were compared by Tukey`s test and consider significant if $p<0.05$ (Snedecor and Conhran, 1991). Statistical analyses were performed by using Costate Computer Software, Version 6.303.

Table I: Experimental diet (%) of organic acids supplemented diet.

Ingredients	OA1	OA2	OA3	OA4	OA5
Fish meal	35	35	35	35	35
Sunflower meal	15	15	15	15	15
Soybean meal	15	15	15	15	15
Corn gluten meal	13	13	13	13	13
Fish oil	10	8	8	8	8
Wheat flour	10	10	10	10	10
Mineral mixture*	1	1	1	1	1
Vitamin premix**	1	1	1	1	1
Malic acid	-	2	-	-	-
Citric acid	-	-	2	-	-
Formic acid	-	-	-	2	-
Lactic acid	-	-	-	-	2
Total	100	100	100	100	100

*Each Kg mineral granules contains: Ca (calcium), 155gm; Mn (manganese), 2000mg; P (phosphorous), 135gm; Cu (copper), 600mg; Mg (magnesium), 55gm; Co (cobalt), 40mg; Fe (iron), 1000 mg; I (iodine), 40mg; Zn (zinc), 3000 mg; Se (selenium), 3mg; Na (sodium), 45gm. **Each Kg of Vitamin premix contains: Vitamin A, 15 M.I.U.; Vitamin D3, 3 M.I.U.; Nicotinic acid, 25000mg; Vitamin B1, 5000 mg; Vitamin E, 6000 IU; Vitamin B2, 6000 mg; Vitamin K3, 4000 mg; Vitamin B6, 4000 mg; Folic acid, 750; Vitamin B12, 9000 mcg; Vitamin C, 15000mg; Calcium pantothenate, 10000mg.

Results and Discussion

Effects of supplementation of different organic acids on Zn, Cu, Fe and Mn contents (µg/g) in whole body of *L. rohita* fingerlings are given in Table I. Results showed that acidification of diet significantly (p<0.05) enhanced the Zn, Cu, Fe and Mn contents in the whole body of *L. rohita* fingerlings. Maximum Effects of supplementation of different organic acids were observed with citric acid supplemented diet on Zn, Cu, Fe and Mn, while minimum effects were recorded in diet containing lactic acid on Zn, malic acid on Cu and Fe and lactic acid on Mn.

Table II: Effects of different organic acids supplementation on Zn, Cu, Fe and Mn contents (µg/g) in *Labeo rohita* fingerlings.

Diet	Organic acid	Zn (µg/g)	Cu (µg/g)	Fe (µg/g)	Mn (µg/g)
OA1	Control	7.776^{e}	3.976^{d}	28.430^{d}	13.136^{d}
OA2	Malic acid	8.520^{b}	4.125^{c}	29.387^{c}	14.294^{c}
OA3	Citric acid	8.946^{a}	4.275^{a}	31.763^{a}	16.095^{a}
OA4	Formic acid	8.303^{c}	4.202^{b}	30.380^{b}	15.152^{b}
OA5	Lactic acid	7.908^{d}	4.172^{bc}	30.215^{b}	14.165^{c}
PSE		0.017	0.016	0.105	0.113
ANOVA		**P-value**			
Organic acids		0.0000*	0.0003*	0.0000*	0.0001*

Means values within columns having different superscripts are significantly different at p<0.05. Data are means of two replicates. PSE is pooled SE= $\sqrt{MSE/n}$ (where MSE is mean-squared error).

The addition of organic acid in formulated feed reduces pH in the fish gut, because the level of acid secretion in fish gut is low in compared to mammals. This reduction in pH value increases phytate hydrolysis; pathogen is killed, lowers the rate of emptying of gastric and improves the absorption of minerals and other nutrients (Shah *et al*., 2015). The present work was studied for determination of the trace mineral contents in *L. rohita* fingerlings in fed acidified diet. Result shows that the supplementation of organic acids enhanced the body mineralization in *L. rohita* fingerlings. Maximum body mineralization was observed in the diet OA3 containing citric acid while minimum mineralization was regarded in the diet OA5 containing lactic acid as compared to other diets with organic acid supplemented. In conclusion citric acid showed best responses as compared to other dietary supplemented organic acid. Our results are similar with the results of Sarker *et al*. (2005) and Vielma *et al*. (1999) who studied the rainbow trout and red sea bream, respectively According to Pandey and Satoh (2008) the calcium, phosphorous and zinc contents increased in rainbow trout with supplemented citric acid diet. Similarly, in another series of experiments, improved minerals contents in bones and whole body of *L. rohita* fed with diet supplementing 3% citric acid was also reported (Baruah *et al*., 2005). The 3% citric acid in *Pagrus major* feed increased the mineral contents (P, Ca, K, Cu, Mn and Fe). Contrast to our results were also observed in red sea bream (Hossain *et al*., 2007), rainbow trout (Pandey and Satoh, 2008) and yellowtail (Sarker *et al*., 2012) because it indicate that non-significant variation of minerals in fishes whole body.

Citric acid acidified soybean meal based diet significantly increase the phosphorous and calcium contents in *Huso huso* as compared to control (Khajepour and Hosseni, 2010). Intestinal brush border membrane has Na^{+}-dependent transport channels for di- and tri-carboxylic acids transportation across the membrane. According to Wolffram*et al*. (1990) and (1992) these channels are also responsible for organic acids are absorption in intestine. Phytate molecule has ability to bind with some minerals including calcium (Erdman, 1979).

Citric acids has unique character of chelating minerals and other element, and minimize the effect of phytase with binding other molecules, due to this activity it provides more susceptible and less stable endogenous phytase (Baruah *et al*., 2005). Intestinal pH low, P and and phytase solubility improved and P and other phytate chelate minerals absorption improves in the intestine. The supplementation of organic acid is lower down the intestinal pH and many cations also bind with intestine and works as a chelating agent (Jongbloed, 1987).

A result from present study also suggests that organic acids increased the mineral consumption in fishmeal and plant based diet saves the inhibitory action in dietary component. Phytate and tricalcium phosphate considered as mineral inhibitors, these inhibitors present in plant and animal protein sources, these sources used for feed formulation. Citric acid has capacity to released adequate inorganic P from tricalcium phosphate and it proves good for fish growth. The correct mechanism of citric acid and many other organic acids in the present study is not known (Pandey and Satoh, 2008). In finale, citric acid showed best responses, while lactic acid showed minimum result as compared to other dietary supplemented organic acid. It provides beneficial and least cost economic diet for fish.

Conflicts of interest

The authors declare no conflicts of interest.

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