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Whole Body Antioxidant Status of Silver Carp Fingerlings Fed Diet Containing Various Dietary Organic Acids

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ABSTRACT

A 10-week feeding trial was conducted to evaluate the whole- body antioxidant status of silver carp fingerlings when fed with diets containing various organic acids. Five experimental diets were formulated, such as D_1 containing no supplemented organic acids, while D_2 contain (2%malic acid), D_3 (2%citric acid), D_4 (2% formic acid) and D_5 (2% lactic acid). During the experiment, water quality parameters including temperature, pH and DO were controlled. Results showed that acidification of diet significantly reduced the activity of SOD, CAT and GPX throughout the body of silver carp fingerlings. Moreover, among different organic acid groups, the maximum value was observed in citric acid while, minimum value was recorded in formic acid. Data on whole -body antioxidant enzymes were subjected to one-way analysis of variance following Steel *et al.* (1996). Differences between among means were compared by Tukey`s Honestly Significant Difference Test and considered significant at ($p<0.05$) (Snedecor and Conhran, 1991).

INTRODUCTION

The global aquaculture industry currently produces 45% of all seafood that is consumed all over the world. It has been estimated that it will be increased by 75% in the next 20 years (FTU, 2007). In Egypt, aquaculture accounts for 60% of total fish production sources (GAFRD, 2007). Essential nutrients are not enough to fulfill the demand of the high quality feeds so complementary feed additives are needed to improve efficiency of feed utilization and survival rates of fish.

Among them, short chain organic acids are mainly used as they have beneficial effects on feed preservation and utilization (Luckstadt, 2006; Atapattu and Senevirathne, 2013; Sing *et al.*, 2014). Organic acid supplementation in fish diet reduces the gastric pH (Baruah*et al.*, 2005) which leads to increase the absorption of nutrients (Boling- Frankenbach*et al.*, 2001) and also help in the breakdown of phytate (Jongbloed, 1987), so it is easily available to fish. Moreover, it also decreases the gastric emptying rate by dietary acidification (Mayer, 1994). Furthermore, it also improves the gut health of animal as they have antagonistic effect on microbes (Ravindran and Kornegay, 1993; Partanen and Mroz, 1999).

Organic acid increases the availability of dietary minerals through acidification in several ways. Firstly, by the modification of mineral transport mechanism by changing the stomach acidity. Secondly, supplementation of organic acid in the diet acts as a chelating agent and affects the complex forming ability of elements (Ravindran and Kornegay, 1993). Thirdly, the absorption area for minerals is increased (Baruah *et al.*, 2007a) through the proliferation of the epithelial cells in gastrointestinal mucosa (Sakata *et al.*, 1995) by the inclusion of organic acid in the diet.

CA, one of organic acid is widely used for acidification of diet as it has unique flavor and high buffering capacity (Hossain *et al.*, 2007). It has a great potential to reduce the antagonistic interactions between trace elements by their chelating effect and also enhances the absorption of other trace minerals (Sugiura *et al.*, 1998).

Proteolytic enzymes are stimulated by CA which leads to increase the feed intake, reduces the activity of microbes including ammonia and also has the capacity to reduce the risks of subclinical infections (Chowdhury *et al.*, 2009; Ou *et al.*, 2013).

Objectives of this study

The aim of present study was to investigate the whole body antioxidant status of silver carp fingerlings fed acidified diets

MATERIALS AND METHODS

Experiment was performed in Fish Nutrition Laboratory, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad.

Fish and experimental condition

Before onset the experiment, silver carp fingerlings were obtained from Government Fish Seed Hatchery, Faisalabad. For acclimatization to indoor conditions, fish were placed for two weeks in tiled tanks (1000 L). During this period basal diet was given for 6 days (Allan & Rowland, 1992) to fish. For feeding trial, 9 species of fish with same initial weight (3.526±0.0056 g) were kept in V shaped tanks (70L). Throughout this time, fingerlings were fed of that basic food once a time in a

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day. For each test diet experiment repeated three times. Feeding trial sustained for two months. During the study period, Jenway pH meter 3510 and DO 970 were used to monitor changings in water quality, specially dissolved oxygen, temperature and pH. All water tanks are inflated by capillary system around the clock.

Feed Ingredients and Experimental Diets

Feed Ingredients and feed stuff obtained from local poultry market and composition of chemical analysis was done by using standard methods (AOAC, 1995). Before incorporating feeding trial, the constituents of food were crushed and filtered to achieve the desired particle size (Table I).

The method for pretreatment of ingredients, 1 kg of the ground constituents such as fish meal, wheat flour, sunflower meal, corn gluten meal and 1.5L of distilled water was adding for made paste and retained it for 38 °C for 16 h and then dried. All dry ingredients were mixed for 15 minutes in an electric mixture. Though continuously stirring, then gradually add mineral mixture, vitamin premix and fish oil.

Five trial diets were made by using various supplementing organic acids at level of 2%. The D_1 contains no supplemented organic acids, while D_2 contain malic acid, D_3 citric acid, D_4 formic acid and D_5 lactic acid, correspondingly. To prepare appropriate dough for each trial feed, slowly mix 10% to 15% water. To make floating particles, then further process it via a laboratory extruder. After particles are dried, they are crushed and sieved to the desired size. Keep the pellets in the refrigerator at -18 ° C until the feeding test is completed.

Table 1: Formulation (%) of feed ingredients

Ingredient	Percentage
Fishmeal	25
Sunflower meal	20
Soybean meal	10
Corn gluten meal	15
Fish oil	7
Rice Polish	10
Wheat flour	9
Mineral mixture**	1
Vitamin premix*	1
Organic acid	2
Total	100

Feeding Procedure and Sample Collection

For experimental feeding trial, the fingerlings were fed of their suggested diet. After feeding time of three hours, the extra food was exhausted by opening the tank valve. Wash water tank thoroughly to eliminate particles from the food then fill-up the water. Afterwards, return the fish to the fish tank. After the two-hour interval, feces were collected in beaker by opening valves of tank. In an oven at 60°C, each of the repeatedly processed feces was dried. Then ground and stored for chemical inquiry. For each repeated sample, the trial was continued to collect 5 g of feces.

Chemical analysis of feed

With help of pestle and mortar fish samples and diet were standardized Methods for determining moisture was: drying in an oven at 105°C for 12 hours, micro Kjeldahl apparatus used for measuring crude protein. By Soxhlet system, crude fat through petroleum ether extraction method determined (Bligh & Dyer, 1959) and in an electric furnace for 12 hours crude fiber measured (Table II)

Table 2: Chemical composition (%) of experimental diet

Diet	Organic acids	DM (%)	CP (%)	CF (%)	Ash (%)
D_1	Control	89.49	31.11	9.05	9.95
D_2	Malic acid	90.1	30.51	9.1	9.62
D_3	Citric acid	90.17	31.071	9.045	9.8
D_4	Formic acid	89.63	31.12	8.94	9.78
D_5	Lactic acid	90.11	30.84	9.16	9.86

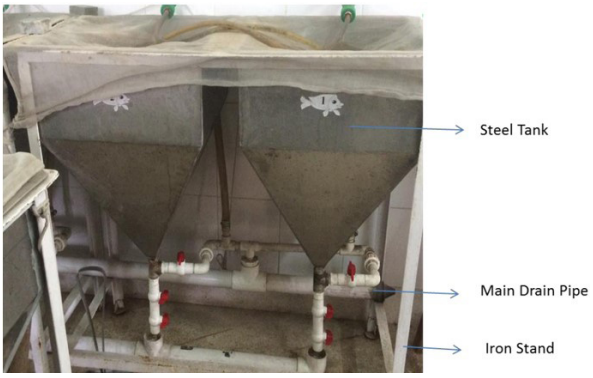

Figure 1: Modified UA system showing steel tank and main pipe

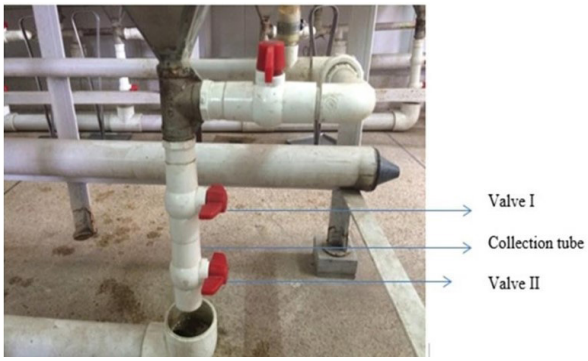

Figure 2: Modified UA System showing valve I and valve II and collection tube

Determination of antioxidant enzymes

Preparation of enzyme extract

Whole body was taken and rinsed with phosphate buffer of pH 6.5 (0.2 M) and homogenized in cold buffer (1:4 w/v) using a blender. After the homogenization, organ homogenates were centrifuged for 15 minutes at 10,000 rpm and 4°C. After centrifugation process, clear supernatants were stored at 80°C for enzyme assay while residue was discarded.

Enzyme Assay of Superoxide dismutase

The activity of superoxide dismutase was determined

by measuring its ability to inhibit the photo reduction of Nitrobluetetrazolium (NBT) following the method of Giannopolitis and Ries (1997).

Procedure

1 ml buffer was taken in cuvette as blank and inserted into spectrophotometer to note the readings of blank, after taking reading spectrophotometer was adjusted at zero at A560 nm. Then 5-6 cuvettes were taken and set them in a light box with an internally mounted light bulb of 30 Watt. Firstly 1 ml of buffer was added to each cuvette, then 0.05 ml enzyme extract and 0.016 ml of riboflavin was added in each cuvette. All the cuvettes were incubated in light box for 12 minutes. The cuvettes were transferred to the spectrophotometer, where 0.067 ml of EDTA/NaCN solution and 0.033 ml of NBT was added to the illuminated reaction mixture. The absorbance was noted after 20 s of reaction. Activity of superoxide dismutase was determined by measuring the % age inhibition of NBT.

$$\%age\ inhibition = \frac{Blank\ (Abs) - Sample\ (Abs)}{Blank\ (Abs)} \times 100$$

Enzyme Assay of Catalase and Glutathione Peroxidase

The activity of peroxidase was determined by measuring its ability to reduce the concentration of H_2O_2 at A470 nm (Civello *et al.*, 1995).

Procedure

A cuvette containing the 2 ml of blank solution was inserted into the spectrophotometer and set it to zero at wavelength of 240 nm. Then a cuvette containing buffered substrate solution was put into the spectrophotometer and initiation of reaction was occurred by adding 0.05 ml of enzyme extract the initiation of reaction was occurred. The reaction time is 3 minutes and noted the absorbance after interval of 1 minute.

Calculation

$$\text{Activity } (Units/ml) = \frac{\Delta A/min \times dilution\ x\ 2\ ml}{0.04 mM^{-1}\ cm^{-1} \times 0.05\ ml}$$

Statistical Analysis

Finally, data regarding the activities of antioxidant enzymes of whole body were subjected to one-way analysis of variance following Steel *et al.* (1996). The differences among means were compared by Tukey`s Honestly Significant Difference Test and considered significant at ($p<0.05$) (Snedecor and Conhran, 1991). Costate Computer Software, Version 6.303 was used for statistical analysis.

RESULTS

Superoxide dismutase status (U/mg protein) in whole body of silver carp fingerlings

Effect of different organic acids on whole body superoxide dismutase status in silver carp fingerlings is shown in table III. Data showed that acidification of diet significantly decreased the activity of SOD in whole body of silver carp fingerlings. Furthermore, maximum value was observed in formic acid while, minimum value was recorded in malic acid among different organic acid groups.

Table 3: Effect of supplementation of different organic acids on superoxide dismutase status (U/mg protein) in whole body of silver carp fingerlings

Diet	Organic acid	SOD
D_1	Control	4.55^a
D_2	Malic acid	3.65^c
D_3	Citric acid	3.83^{bc}
D_4	Formic acid	4.10^b
D_5	Lactic acid	4.02^b
PSE		0.068
P-value		0.0018**

Data are means of three replicates, P<0.05 Organic acids

PSE = pooled SE = √MSE/n (where MSE= mean-squared error)

Catalase status (U/mg protein) in whole body of silver carp fingerlings

Effect of different organic acids on whole body catalase status in silver carp fingerlings is shown in table IV.Data showed that acidification of diet significantly decreased the activity of CAT in whole body of silver carp fingerlings. Furthermore, maximum value was observed in malic acid while, minimum value was recorded in lactic acid among different organic acid groups.

Table 4: Effect of supplementation of different organic acids on catalase status (U/mg protein) in whole body of silver carp fingerlings

Diet	Organic acid	CAT
D_1	Control	65.66^a
D_2	Malic acid	58.52^b
D_3	Citric acid	53.22^c
D_4	Formic acid	50.72^c
D_5	Lactic acid	50.05^c
PSE		1.427
P-value		0.0025**

Data are means of three replicates, P<0.05 Organic acids

PSE = pooled SE = √MSE/n (where MSE= mean-squared error)

Glutathione peroxidase status (mU/mg protein) in whole body of silver carp fingerlings

Effect of different organic acids on whole body glutathione peroxidase status in silver carp fingerlings is shown in table V. Data showed that acidification of diet significantly decreased the activity of GPX in whole body of silver carp fingerlings. Furthermore, maximum value was observed in citric acid while, minimum value was recorded in formic acid among different organic acid groups.

Table 5: Effect of supplementation of different organic acids on glutathione peroxidase status (mU/mg protein) in whole body of silver carp fingerlings

Diet	Organic acid	GPX
D_1	Control	75.82[a]
D_2	Malic acid	70.11[b]
D_3	Citric acid	71.46[b]
D_4	Formic acid	68.27[b]
D_5	Lactic acid	71.39[b]
PSE		0.904
P-value		0.0149*

Data are means of three replicates, P<0.05 Organic acids
PSE = pooled SE = √MSE/n (where MSE= mean-squared error)

DISCUSSION

Currently, there is considerable interest in the commercial use of organic acids in fish diets, both to control disease and to enhance growth performance. Researches have reported that several organic acids, their salts or mixtures thereof can improve growth, feed utilization and disease resistance in fish (Baruah *et al* 2007; Hossain, Pandey & Satoh 2007; Sarker, Satoh & Kiron 2007; Luckstadt 2008). Hormones, antibiotics, ionospheres and some salts are most commonly used as growth promoters to increase the productive performance of fish (Fuller, 1992; Go´ngora, 1998; Klaenhammer and Kullen, 1999). Antibiotic growth promoters are used in fish diets as feed additives to favor growth and environment friendly aquaculture. However, it is necessary to make use of these antibiotic growth promoters in less amount as feed additive in fish diets worldwide to enhance the development of cross resistances to humans. However, the supplementation of organic acid is beneficial to replace the feed additives.

(Kim *et al.* 2006) investigated that organic acid supplemented diet can improve the growth and performance of fishes. Fish feed industry has been using organic acids for decades where most extensively used acidifiers contain lactic, citric, fumaric and formic acid.

(Baruah *et al.* 2007) designed a factorial experiment to study the effect of dietary microbial phytase, citric acid and their interactions on growth performance of Labeo rohita juveniles. Hence, it is suggested that phytase and citric acid act synergistically to improve growth performance and nutrient digestibility of L. rohita juveniles.

(Hossain *et al.* 2007) conducted a feeding experiment to investigate the effects of organic acids on the growth and phosphorus utilization in fish. Therefore, it can be concluded from these results that organic acids can play an important role in development of highly required eco-friendly diets.

(Pandey & Satoh 2008) observed phosphorus (P) and nitrogen (N) retention in rainbow trout fed on low fish meal based diets supplemented with organic acid. Hence, results conclude that low fishmeal based diets may prove beneficial for growth of rainbow trout if supplemented with certain organic acids.

Phromkunthong *et al.* (2010) determined the combined effect of citric acid and microbial phytase on phosphorus utilization. Cyprinus carpio fingerlings were treated with citric acid and phytase for 60 days. It can be concluded that positive effects of phytase can be achieved by adding low dose of citric acid.

CONCLUSION

Findings of experiment are as follows:

- Supplementation of organic acids significantly ($p<0.05$) decreased the whole body superoxide dismutase activity (U/mg protein) in silver carp fingerlings.
- Supplementation of organic acids significantly ($p<0.05$) decreased the whole body catalase activity (U/mg protein) in silver carp fingerlings.
- Supplementation of organic acids significantly ($p<0.05$) decreased the whole body glutathione peroxide activity (mU/mg protein) in silver carp fingerlings.

In conclusion, supplementation of organic acids decreased the antioxidant enzymes in silver carp fingerlings.

Authors Contribution

K.R planed, did experiments and wrote manuscript. T.D, S.A, M.A and S.A helped in experimental work.

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