Pakistan J. Zool., vol. 56(3), pp 1243-1247, 2024 **DOI**: https://dx.doi.org/10.17582/journal.pjz/20200507090510

A Study of Micro-Mineral Retention in Silver Carp Fingerlings Fed Acidified Diet

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Article Information
Received 07 May 2020
Revised 18 September 2022
Accepted 08 October 2022
Available online 14 February 2023 (early access)
Published 12 April 2024

Authors' Contribution
KR planned, did experiments and wrote manuscript. MA, SA, AI and FM helped in experimental work.

Key words
Silver carp fingerlings, Organic acids, Micro-minerals, Retention and excretion

ABSTRACT

An experiment was conducted to examine trace minerals (Zn, Cu, Fe and Mn) retention in silver carp fingerlings when fed with diet containing various organic acids. Five experimental diets such as D1 contains no supplemented organic acids, while D2 contain (malic acid 2%), D3 (citric acid 2%), D4 (formic acid 2%) and D5 (lactic acid 2%) were formulated. The feeding trial time was eight weeks. Throughout experiment, water quality parameters comprising of temperature, pH and DO was checked. Results showed that organic acid supplementation increase concentration of trace elements retention in silver carp fingerlings. The minimal activity retained by trace elements was observed in diets containing lactic acid. The results presented that best responses showed lactic acid in comparison of other dietary supplemented organic acids.

INTRODUCTION

The important basis of protein in fish feed formulation is fishmeal because it contains important nutrients (Zhou *et al*., 2004). Mineral content of the diet absorbs in terrestrial animals increased by addition antibiotics (Ravindran *et al*., 1984; Radecki *et al*., 1988; Windisch *et al*., 1994), phytase (Adeol *et al*., 1995) and organic acids (Ravindran and Kornegay 1993). In reaction to organic acids supplementation, better manufacture of Rainbow trout (Sugiura *et al*., 1998), Red sea bream (Sarker *et al*., 2005) and Rohu (Baruah *et al*., 2005) have been stated in few available studies.

The supplementation of fish feed with antibiotic growth promoters rise the growth, survival rate of fish and the conversion of feed. However, the micro-biota of fish becomes more resistant with the use of these antibiotics, consequently, producing the risk of cross-resistance among human. Due to these public anxieties, usage of antibiotic growing promoters in aquaculture has been banned throughout the world.

Accordingly, alternative growth enhancing condiments e.g., essential oils, probiotics, herbs, enzyme and organic acids are being focused by researchers. However, the short-chain organic acids play a vital role in preservation of feed which makes them more important among others (Sing *et al*., 2014).

Presently, there is important concern in commercial usage of organic acids in diets of fish for control of diseases and increase performance in growth (Baruah *et al*., 2007; Hossain *et al*., 2007). Although nutritive use of organic acid supplements has been reported to be better but the growth promoting effects of organic acid supplements have been reported to be contradictory, which seems to be the kind of organic acid verified as studied by (Luckstadt, 2008). Among these biological acids for nutrition acidification, citric acid has been completely eliminated due to its exclusive taste and extraordinary defense ability (Hossain *et al*., 2007).

Acidification by organic acids can greatly affect the bio-availability of nutritional minerals in a variety of ways. First, changes in gastric acidity lead to alterations in mineral carrying mechanisms. Second, diet containing organic acids supplementation reduces the ability of complex formation and chelation of the elements (Ravindran and Kornegay, 1993) because organic acids form a chelate with Ca. Therefore, the antagonism between phosphate and calcium or trace minerals are inhibited at the brush border

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0030-9923/2024/0003-1243 $ 9.00/0

of intestine, thereby ensuring increased the assimilation of other trace minerals and phosphorus (Sugiura *et al*., 1998). Third, proliferation of gastrointestinal mucosal cells stimulated by organic acids (Sakata *et al*., 1995), thus mineral absorption increased (Baruah *et al*., 2007).

In this study, we examined micro-mineral retention in silver carp fingerlings fed different organic acids diet and also excretion was checked.

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 and 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 (70 L). Throughout this time, fingerlings were fed of that basic food once a time in a 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 min 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 D1 contains no supplemented organic acids, while D2 contain malic acid, D3 citric acid, D4 formic acid and D5 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 I. Chemical composition (%) of experimental diet.

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

Feeding procedure and sample collection

For experimental feeding trial, the fingerlings were fed of their suggested diet. After feeding time of three h, 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 h, micro Kjeldahl apparatus used for measuring crude protein. By Soxhlet system, crude fat through petroleum ether extraction method determined (Bligh and Dyer, 1959) and in an electric furnace for 12 h crude fiber measured (Table II).

Analysis of minerals

To approximate the micro-mineral, the sample of 1 gram (trial diet and fish body) was weighed and take into a conical flask and added 30 ml of nitric acid and on a warm plate put the flask. Once composition activates boiling then added 10 ml of perchloric acid then again place the flask on a warm plate and heated till 1 ml of the blend

remains. Removed flask and dilute to 100 ml via addition of distilled water to convert most of it into transparent crystals. By utilizing filter paper, this absolute volume is filtered in order to eliminate all grainy matter in the solution of digestion earlier to study of minerals (AOAC, 1995). Afterward proper dilution using atomic absorption spectrophotometer minerals contents were estimated.

Table II. Formulation (%) of feed ingredients.

Diet	Organic acids	DM (%)	CP (%)	CF (%)	Ash (%)
D1	Control	89.49	31.11	9.05	9.95
D2	Malic acid	90.1	30.51	9.10	9.62
D3	Citric acid	90.17	31.07	9.04	9.80
D4	Formic acid	89.63	31.12	8.94	9.78
D5	Lactic acid	90.11	30.84	9.16	9.86

Table III. Retention of trace elements in silver carp fingerlings fed acidified diet.

Diet	Organic acid	Zn retention	Cu retention	Fe retention	Mn retention
D1	Control	73.39^{e}	26.28^{d}	65.55^{e}	65.07^{e}
D2	Malic acid	90.76^{b}	33.60^{b}	81.21^{b}	80.16^{b}
D3	Citric acid	96.87^{a}	35.83^{a}	86.77^{a}	85.69^{a}
D4	Formic acid	84.70^{c}	31.55^{c}	75.95^{c}	74.56^{c}
D5	Lactic acid	79.00^{d}	30.09^{c}	70.69^{d}	70.45^{d}
PSE		1.22	0.54	1.04	1.06
ANOVA					
P-value		.0002 ***	.0004 ***	.0002 ***	.0002 ***

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

Statistical analysis

Experiment was performed in a completely randomized design. With help of one-way analysis of variance, statistical analysis was done to examine retention and excretion data of trace minerals (Steel *et al*., 1996). Significant or non-significant response of these factors can be confirmed by p-value of one-way analysis of variance (Table III).

$$\text{Retention (\%)} = \frac{\text{Final nutrient content} - \text{Initial nutrient content}}{\text{nutrient intake}} \times 100$$

$$\text{Excretion } \left(\frac{\text{kg}}{\text{t}}\text{production}\right) = \frac{[\text{FCR} \times \text{Nutrient in diet(kg)} - \text{Nutrient retainedin fish(kg)}]}{\text{production(kg)}} \times 1000$$

RESULTS

Organic acids retention (%) in silver carp fingerlings

Effect of various organic acids in silver carp fingerlings is given in (Table III). Data presented that concentration organic acid retention in silver carp fingerlings significantly increase by acidification of diet. However, maximum value was seeing in citric acid while lowest value was recorded in lactic acid among different organic acids groups.

Organic acids excretion (%) in silver carp fingerlings

Effect of diverse organic acids in silver carp fingerlings is given in (Table IV). Data showed that acidification of diet considerably reduction the concentration of organic acid excretion in silver carp fingerlings. Furthermore, greatest value was perceived in lactic acid while minimum value was noted in citric acid among different organic acids groups.

Table IV. Excretion of trace elements in silver carp fingerlings fed acidified diet.

Diet	Organic acid	Zn excretion	Cu excretion	Fe excretion	Mn excretion
D1	Control	0.051^{a}	0.025^{a}	0.11^{a}	0.028^{a}
D2	Malic acid	0.01^{d}	0.017^{d}	0.04^{d}	0.012^{d}
D3	Citric acid	0.007^{e}	0.015^{e}	0.02^{e}	0.007^{e}
D4	Formic acid	0.02^{c}	0.019^{c}	0.06^{c}	0.017^{c}
D5	Lactic acid	0.03^{b}	0.021^{b}	0.08^{b}	0.022^{b}
PSE		0.002	0.0005	0.004	0.001
ANOVA					
P value		.0002 ***	.0002 ***	.0002 ***	.0002 ***

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

DISCUSSION

The chief source of nutrition is minerals that cause contamination of freshwater bodies. Consequently, it is compulsory to control pollution in environment via decreasing the content of mineral in the diet and the mineral discharges in the feces. In fish, numerous investigations have been accompanied to study irritating nurturing impacts of different materials comprising lipids, nucleic acids, some extracts from other animals and amino acids (Adams *et al*., 1988; Sidorov, 1995; Kasumyan and Morsi, 1996).

Extracts from fish and other aquatic organisms have high levels of organic acids (Hidaka *et al*., 1992). Some investigations had revealed their positive impacts on fish (Adams *et al*., 1988; Hidaka *et al*., 1992). Certain organic acids, particularly citric acid, methoxyacetic acid and acetic acid are likewise additional to the pellets for storing and progress feed application (Kumar *et al*., 1997; Sugiua *et al*., 1998). From pellets of diet, these acids may filter

and affect the feeding activities of fish.

Previous studies described that development of red sea bream influence stimulated via in form of chemical of trace minerals. The digestibility of nutrients and energy in pigs improve by organic acid. The better-quality presentation and digestibility as pretentious by organic acid is considered to be caused by: (i) Dropped pH subsequent in a developed dissociation of mineral composites (ii) reduced rate of gastric emptying; and (iii) establishment of chelated mineral complexes, which are effortlessly fascinated.

In recent analysis, nutritional CA resulted innovative conservation of trace elements in body of fingerlings. Meanwhile, excretion of this mineral was mainly low in comparison of control diet in silver carp fingerlings fed. In rainbow trout, better retention of P and reduced P loading via addition of CA (1%) in low fish meal initiated food were also observed (Hernandez *et al*., 2013). Alike consequences were perceived by CA accumulation to food in broilers (Demirel *et al*., 2012). Xie *et al*. (2003) described that lactic acid at a dietary concentration of 0.01–0.0001 M encouraged the feeding behavior of Tilapia nilotica. Nonetheless, such a positive influence of lactic acid was not observed in the current study as the feed consumption (g/fish) was the lowest in the LA diet.

CONCLUSION

In conclusion, organic acids supplementation increased the mineral retention and decreased their excretion in the silver carp fingerlings. The results presented that best responses showed lactic acid in comparison of other dietary supplemented organic acids.

ACKNOWLEDGMENT

Authors are grateful to Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan for valuable support in carrying out this work.

Funding

The study received no external funds.

IRB approval

The protocols and procedure of this study were approved by Animal Use and Animal Care Committee of Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad. Pakistan.

Ethical statement

The animal study was reviewed and approved by Fish Nutrition Laboratory, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad.

Statement of conflict of interest

The authors have declared no conflict of interest.

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