## Citric Acid as a Feed stimulant

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Utility of citric acid as a feed stimulant for fry of *Ctenopharyngodon idella* was tested. The fry ate more feed containing dry aquatic macrophyte when citric acid was added. Food consumption, feed conversion efficiency and specific growth rate were higher at 0.01 M citric acid concentration.

Key words: Citric acid, feed stimulant, Ctenopharyngodon idealla.

The high cost and scarcity of fishmeal have created problems for aquaculture feed industry in recent years. Several alternate sources for protein in fish feeds have been tried for culture fishes. In predominently herbivorous fishes, plants and agricultural by-products have been used as partial replacements for fishmeal (Johnsen & Adams, 1986).

However, incorporation of plant products in dry form adversely affects the acceptability and digestibility of these feeds (Rhoades, 1979; Adams & Johnsen, 1986; Adams et al., 1988). Addition of certain substances to the feeds creates acceptability and enhances food intake in fishes (Linstedt, 1971; Rhoades, 1979; Tandler et al., 1982; Adams & Johnsen, 1986. Amino acids, organic acids like citric acid (Adams et al., 1988; Shaiu & Chou, 1994), enzymes (Carter et al., 1994), natural products (Meena Kumari, Pers. comm.) etc. are some such substances. The grass carp, Ctenopharyngodon idella is a herbivorous culture fish whose fry feed on zooplankton and even on fish larvae (Zonneveld & Zon, 1985). Hence in hatcheries the fry are fed with fishmeal

based diets. In large hatcheries, rearing fry for longer periods becomes sometimes necessary and feeding fishmeal based diets would be uneconomical. Offering feeds containing plant products reduces feed cost by decreasing fishmeal content in the fry feeds. In the herbivorous fish Tilapia zilli, citric acid, an organic acid found in citrous group of fruits was found to enhance food consumption (Adams et al., 1988) but its role in increasing feed efficiency and growth is not known. In the present study, the effect of incorporation of citric acid to a formulated feed containing dry aquatic macrophyte as partial replacement of fishmeal on the utilization of diet and growth of the fry of C. idella is investigated.

## Materials and Methods

Grass carp fry obtained from a commercial hatchery were used in this study. They were transported to the laboratory and acclimatized to the experimental conditions for two weeks. The fry were stocked in large 1000 l fiber glass tanks with facilities for aeration and water exchange. They were initially fed with a fishmeal based diet.

Four practical experimental diets were formulated to provide graded levels of citric acid to the fish. A control feed and 0.01 M, 0.02 M and 0.03 M citric acid incorporated feeds were prepared using ingredients like groundnut oil cake, rice bran, fish meal, Bengal gram powder and dried powdered aquatic macrophyte (Ceratophyllum demersum). Ingredients of the experimental diets are given in Table 1. The ingredients were dried powdered and seived through 100 um mesh sized seive. The powdered ingredients were mixed with water (50%) to a dough and cooked in a pressure cooker for 30 min. After cooling, powdered aquatic macrophyte, vitamins, mineral mixture and citric acid at specific concentrations were added. The feed was mixed thoroughly and the dough was passed through a pelletizer (0.5 mm diameter) and dried at 60°C for 24 h in

Table 1. Ingredients used in preparation of feeds for different treatments (for 1 kg)

Ingredients	Feed I (g)	Feed II (g)	Feed III (g)	Feed IV (g)
Groundnut oil cake	300	300	300	300
Rice bran	250	250	250	250
Fish meal	200	200	200	200
Powdered aquatic macrophyte	150	150	150	150
Bengal gram flour	100	100	100	100
Vitamin-mineral mixture	10	10	10	10
Citric acid	-	2.1	4.2	6.3

Composition of vitamin-mineral mixture: Each g contained: Vitamin A 5000 IU, Vitamin D3 200 IU, Vitamin E 7.5 mg, Vitamin B1 2.5 mg, Vitamin B2 2.5 mg, Nictotinamide 25 mg, D - Panthenol 2.5 mg, Vitamin B6 1 mg, Vitamin C 40 mg, Folic acid 500 mcg, Vitamin B12 2.5 mcg, Ferrours fumarate 25 mg, Dibasic calcium phosphate 35 mg, Copper sulphate 0.1 mg, Manganese sulphate 0.1 mg, Zinc sulphate 50 mg, Potassium iodide 0.025 mg, Magnesium oxide 0.15 mg.

a hot air oven. The protein content of all feeds were 25%.

Four fibre glass tanks of 2001 capacity were cleaned, dried and filled with fresh water. Groups of ten fishes were anaesthetised with anaesthetic ether, weighed and released into each of the tanks. Each acquarium was provided with continuous aeration by an air stone connected to an air pump. A water flow of about 1 1/h was also maintained in each tank. Temperature of the water remained at 28±1.5°C and dissolved oxygen near saturation. Three replicates for each treatment were also set up in the same manner. The fry were offered excess quantities of the experimental diets I, II, III and IV respectively (Table 1). The uneaten food particles were recovered after 8 h. The faecal matter accumulated at the bottom of the tank was collected by siphoning and filtering the bottom water through bolting silk of 60-65 um mesh size. The experiment was conducted for 45 days. Food intake was calculated on a daily basis and expressed as mg of food eaten/g fish/day. The assimilation of food was determined by deducting the faecal output from the feed consumed by the fish. Gross conversion efficiency was calculated as increase in weight divided by dry weight of feed consumed. Specific growth rate was estimated as SGR = (log Wf-log Wo)/t. where Wo and Wf were the initial and final weight respectively and t is the number of days. Difference between means of treatments were tested to find out the level of significance by ANOVA.

## Results and Discussion

Data on growth and feed efficiency from all the replicates for each treatment were pooled together (Table 2). Food consumption was higher on diets

Table 2. Feed	utilization	and	growth para	ameters for	C. idella	feeding	on the fo	our expe	rimental diets
Treatment	Initial	Final	Food		Assimila-	Food	Net		Mean daily % incre

	Treatment	Initial weight (g)	Final weight (g)	Food intake (mg/day)	Faecal output (mg/day)	Assimila- tion efficiency	Food conver- sion ratio	Net conversion efficiency	Specific growth rate (mg/day)	Mean daily growth rate (mg/day)	"% increase in average daily weight gain with respect to control
I	Control	0.54 ±0.0141	4.59 ±0.1410	139.2 ±6.55	9.12 ±2.13	93.48 ±1.258	1.422 ±0.0656	7.546 ±0.290	21.3	97.70	
11	0.01M citric acid	0.56 ±0.0384	5.60 ±0.0547	148.2 ±10.47	7.94* ±2.31	94.51 ±2.062	1.327 ±0.0923	8.054 ±0.77	22.1 ±0.604	112.00	14.63
Ш	0.02 M citric acid	0.55 ±0.0228	5.23 ±0.2280	142.0 ±10.70	11.20 ±1.93	91.25 ±1.882	1.367 ±0.1071	8.072 ±0.72	21.70 ±0.360	104.00	6.44
IV	0.03 M citric acid	0.56 ±0.0485	5.19 ±0.0485	143.4 ±10.60	13.40 ±2.14	90.63 ±1.203	1.405 ±0.1046	7.954 ±0.541	21.4 ±0.783	102.08	5.22

<sup>\*</sup> Significant at p 0.01

containing citric acid than control and among the citric acid containing diets, mean food consumption was highest with diet 2. The group fed on diet 2 ate more food, and showed higher absorption, food conversion efficiency and specific growth rate. However, except for faecal elimination, the differences among the groups between other parameters were not statistically different. The addition of citric acid resulted in an increase in food consumption and higher growth efficiency. In the herbivorous fish T. zilli, Adams et al. (1988), found that 0.01 M citric acid induced the fish to high feeding behaviour, but they did not study its growth efficiency.

The acidic substances present in feeds have been reported to be responsible for stimulating food consumption. The low pH of citric acid at 0.01 M (Adams et al., 1988) seems to be responsible for the enhanced food consumption. results also indicate that this concentration may enhance the digestibility and absorption efficiency as evidenced from the significantly lower elimination of faecal matter by the

group. The feeding stimulants are also reported to increase enzyme activity in the alimentary canal which helps in better digestibility of the feed (Takii et al., 1988). Increased food intake, absorption efficiency and digestibility seems to be responsible for the high growth rate of fish fed on diet 2. The 14.6% increase of mean growth rate in this group may be profitable in fish farms relying on supplementary feeds. The suitability of many chemical substances being tested as feed additives and stimulants is doubtful as they may remain as residues in fish tissues and pose hazards to them and also for the consumers. Citric acid being an organic acid present in fruits and also used as a food preservative, can be safely used as feed stimulant in fish farms. Hence, in spite of the superior dietary efficiency of the citric acid treated feed being not statistically significant, the approach merits further investigations.

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