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FEEDING STIMULATORY EFFECTS OF *CYPERUS ROTUNDUS* TUBER ON *CIRRHINUS MRIGALA*

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ABSTRACT

Traditionally tubers of cyperus *(Cyperus rotundus)* and its extracts have been used for alluring fish during harvesting in India. An experiment was conducted to evaluate its feeding stimulatory activity and effect on the growth of a commercially important freshwater fish, *Cirrhinus mrigala*. Three isonitrogenous and isocaloric formulated diets *viz.* plant ingredient based control and control supplemented with cyperus tuber (CS) at 1% and 5% levels were fed to the fingerlings of mrigal, *Cirrhinus mrigala* (2.68 + 0.20 g) for a period of 45 days. The growth performance and the activity of metabolic enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), in liver, gill and muscle tissues of mrigal were studied during every 15 days interval. Highest relative growth (72.28%) was obtained in the mrigal fed with the diet containing 5% cyperus (5% CS), while the relative growths were 66.18% and 43.40% for the fish fed with the 1% CS diet and control respectively. The activities of AST and ALT were significantly higher ($p<0.01$) in both 1% and 5% CS diets as compared to the control in all the tissues studied. Higher aminotransferase activities were observed in the tissues of 5% CS group than in those of 1% CS group throughout the experimental period. The observed higher enzymatic activity was concomitant with the higher growth rate in fish. The results suggested that cyperus tuber supplementation increased feed palatability and growth.

Key words: Feeding stimulants, *Cyperus rotundus*, *Cirrhinus mrigala*, Aspartate aminotransferase, Alanine aminotransferase

INTRODUCTION

Most aquaculture waste originates from the feed, which constitutes a major part of waste output in any fish farming operation (Cho *et al.*, 1991). Consequently, management of aquaculture wastes must be approached through diet formulation, improvement of feed utilization and adoption of feeding regimes designed for specific farming conditions. Very often, feed, when contains high cost animal proteins and vitamins, accounts for more than 40 % of the total expenditure under fish farming practices. Further, one of the thrust areas in fish nutrition has been to replace expensive fishmeal with low-cost

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plant proteins of good digestibility in the diets for aquaculture candidate species. But use of plant proteins, which introduces an alien taste and flavour in the diet, reduces acceptability and palatability of the diet. In this context, use of feeding stimulants assumes significance to improve the utilisation of diets containing low cost feedstuffs of low palatability (Takeda and Takii, 1992). Electrophysiological and behavioural experiments investigating the taste or the olfactory nerves in fish have provided evidence that chemical compounds stimulate fish feeding (Carr *et al.*, 1977; Kohbara *et al.*, 2000). An increase in the dietary palatability results in an increase of satiation amounts and a reduction of feeding time (Ishiwata, 1968 a, b).

Fish is attracted and stimulated to feed by terrestrial vegetation such as the vegetable lettuce *Lactuca sativa var. longifolia* (Johnsen and Adams, 1986), spice anise, *Pimpenella anisum* (Takei, 1967), caraway, *Crarum caruvi* and cumin, *Cuminum cyminum* (Harada, 1990 and 1992). In India, the traditional use of extracts from tubers of Cyperus *(Cyperus rotundus),* tapioca *(Manihot utilissima)* and seeds of drumstick *(Moringa* spp.) pods is well known to allure fish for harvesting purpose. However, the feeding stimulatory activity of *Cyperus* spp. has not been yet established scientifically. Mrigal *(Cirrhinus mrigala),* a freshwater herbivorous fish of commercial importance in the Indian subcontinent, contributes 5.2 lakh t (37%) to the total Indian major carp production in India (FAO, 1999). In view of its growing importance in freshwater fish culture, the feeding stimulatory activity of cyperus was evaluated in mrigal in order to develop a suitable and cheap feeding stimulant from plant source. The effect of cyperus supplementation on the activity of metabolic enzymes viz. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) of the fish was also studied.

MATERIAL AND METHODS

Experimental diet

A practical diet (Table 1) utilizing soybean meal, groundnut oilcake as the primary protein sources was formulated keeping in view the nutritional requirements of mrigal (New, 1987). The test diets were prepared by replacing wheat bran with cyperus tuber powder at a level of 1% and 5% in the control diet. The diets were isonitrogenous and isocaloric. Tubers of *Cyperus rotundus* were obtained from the paddy fields of Khammam, Andhra Pradesh and were thoroughly cleaned with freshwater and sun dried. All the ingredients were properly dried and ground so as to pass through a 0.2 mm mesh size. The entire solid feed ingredients except gelatin, oil, cyperus powder and vitamin and mineral mix were mixed thoroughly in a food mixer. Cyperus tuber powder (CS) was mixed in oil at different concentrations and added to the feed mix. Vitamin, mineral mix and gelatin were mixed with adequate water that was to be blended into the mixture to attain a dough consistency suitable for pelleting. Each diet was pelleted through a 2 mm die by using hand pelletizer. After pelleting, the diets were dried to moisture content of about

Table 1 : Ingredient composition of the test diets, expressed as % dry weight

Ingredients	Diet 1 (Control)	Diet 2 (1% CS)	Diet 3 (5% CS)
Groundnut oil cake	26	26	26
Wheat bran	34	33	29
Wheat flour	13	13	13
Soya bean meal	20	20	20
Gelatin	2	2	2
Sun flower oil	4.5	4.5	4.5
Vitamin* and mineral mix**	0.5	0.5	0.5
Cyperus powder	-	1	5
Total	100	100	100

* Vitamin mixture (mg/100g): beta-carotene - 9.6; D_2 Calciferol - 1.2; Menadione - 1.2; Ascorbic acid - 2000; thiamine HCI - 4; riboflavin - 8; nicotinic acid - 40; pyridoxine HCI - 12; Calcium pantothenate - 60; folic acid - 0.8; p - amino benzoic acid - 10; alpha - tocopherol acetate - 20; Choline chloride - 120; Inositol - 400; Biotin - 0.4; Cyanocobalamine - 0.08.

** Mineral mixture (g/100g): KH_2PO_4 1.6; $CaHPO_4$ 3.44; NaH_2PO_2 .H_2O 0.5; $MgSO_4$.$2H_2O$ 2.46

5% and kept in air tight sealed plastic bags till use.

Experimental animals

Fingerlings of *Cirrhinus mrigala* weighing 2.49 to 2.88 g were obtained from the Government Fish Farm, Khopoli, Maharashtra, and were acclimatized for 10 days to the laboratory condition. Fourteen fingerlings (mean weight ± standard deviation, 2.68 ± 0.2g) were stocked in each of the three 30 *l* circular troughs. The test diets were offered to the three groups of fish. The fish were fed at 7% of the biomass per day. The daily ration was split into 40%, 40% and 20% and offered at approximately 07:00, 14:00 and 20:00 h respectively. Everyday the uneaten feed and the faecal matter were removed in the morning before the first feeding. Complete water exchange was done every alternative day. Aeration was maintained uniformly throughout the experimental period. Water temperature, dissolved oxygen and pH, monitored daily, ranged between 23-28^0 C, 5.2-7.1 mg/l and 7.3-8.2 respectively. Ammonia concentration, monitored once a week, ranged between 0.08-0.18 mg/l. The experiment was carried out for 45 days. Weight of the fingerlings was taken at 15 days interval in order to adjust the feed ration.

Chemical analysis

Feed samples were analyzed for moisture, crude protein, crude fat, crude

ash, crude fibre and nitrogen free extract using procedures described by AOAC (1990).

Determination of enzyme activities

Fish samples of four numbers were taken for enzymatic analysis from the treatment groups on day 15, day 30 and day 45. For sacrificing, the fishes were stunned by giving gentle blow on the head region. The fish were cut open through the anus followed by immediate removal of liver, gill and pectoral muscle in the hierarchy in order to prevent immediate changes in the concentration of examined parameters within the organs or tissues. The tissues were weighed and macerated with 0.25 M chilled sucrose solution in mortar and pestle to prepare 5% of homogenate for muscle and gill tissue, and 1% for liver. The homogenate was centrifuged at 4400 rpm for 15 min. and supernatant was collected and stored in a refrigerator. It was subsequently used for the estimation of aspartate aminotransferase (EC 2.6.1.1) (Wootton, 1964), alanine aminotransferase (EC 2.6.1.2) (Wootton, 1964) and protein (Lowry, 1951). Enzymatic activity was expressed as μmole of pyruvate formed per min. per mg protein.

Statistical analysis

Mean values of all the parameters were statistically analyzed by one way analysis of variance (ANOVA) by using a standard statistical package SPSS (Version 11.0). The difference between any two groups were compared by Duncan's multiple range test.

RESULTS

The proximate composition of the test diets (Table 2) did not vary appreciably. The highest relative growth (72.28%) was obtained with fish fed with the diet containing 5% cyperus (5% CS), while the relative growths were 66.18% and 43.40% for the fish fed with the 1% CS diet and control respectively (Figure 1). In this study, hepatic enzymes viz. aspartate aminotransferase and alanine aminotransferase were studied to understand the protein turnover in different tissues of the fish (Table 3).

Table 2 : Proximate analysis of test diets (g/100g)

Constituents	Diet 1 (Control)	Diet 2 (1% CS)	Diet 3 (5% CS)
Moisture	5.14	5.02	5.09
Crude protein	30.15	29.98	29.80
Crude fat	7.80	7.60	7.58
Crude ash	9.10	9.30	9.19
Crude fibre	1.45	1.60	1.71
Nitrogen free extract	46.36	46.50	46.63

Aspartate aminotransferase

For the first 15 days, the highest enzyme activity of liver (16.12 μ mol. of pyruvate/ mg protein / g tissue/ min) was observed in the fish receiving the diet containing 5% CS than the other groups. It was significantly ($p<0.01$) different than that of the fish receiving the control diet (12 μ mol. of pyruvate / mg protein / g tissue/ min) but not from the 1% CS (15.89 μ mol. of pyruvate / mg protein / g tissue/ min). The enzyme activity of the liver was more in the fish receiving the diets containing cyperus than the control one

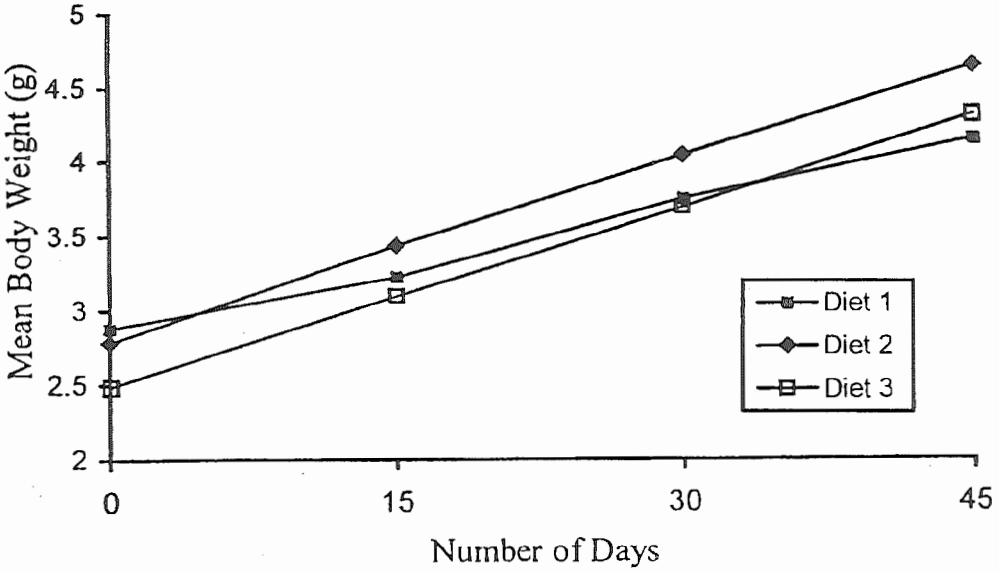

Fig. 1. Growth performance of Cirrhinus mrigala fed diets viz diet 1 (control), diet 2 (1% CS) and diet 3 (5% CS) during the experimental period

Table 3 : Enzyme activity[1] of liver, gill and muscle of *Cirrhinus mrigala* receiving diet 1(control), diet2 (1% CS) and diet 3 (5% CS) during the experimental period.

Period	Enzyme activity/ Feed type	Aspartate aminotransferase (μ mol. of pyruvate/mg protein/ g tissue / min)			Alanine aminotransferase (μ mol. of pyruvate/mg protein/ g tissue / min)		
		Liver	Gill	Muscle	Liver	Gill	Muscle
15 days	Diet 1	12.00 ± 0.40^{ax}	11.13 ± 0.68^{ax}	8.71 ± 0.26^{ax}	11.46 ± 0^{ax}	5.28 ± 0.48^{ax}	8.15 ± 0.51^{ax}
	Diet 2	15.89 ± 0.13^{bx}	17.19 ± 0.21^{bx}	9.47 ± 0.29^{bx}	11.73 ± 0.37^{ax}	6.85 ± 0.51^{bx}	11.42 ± 0.60^{bx}
	Diet 3	16.12 ± 0.51^{bx}	17..87 ± 0.11^{cx}	10.90 ± 0.52^{cx}	18.83 ± 0.14^{bx}	9.81 ± 0.78^{cx}	11.83 ± 0.33^{bx}
30 days	Diet 1	12.49 ± 0.67^{ax}	9.14 ± 0.34^{ay}	8.65 ± 0.46^{ax}	14.33 ± 0.35^{ay}	7.72 ± 0.48^{ay}	5.28 ± 0.48^{ay}
	Diet 2	13.53 ± 0.72^{ay}	16.27 ± 0.15^{by}	11.20 ± 0.23^{by}	16.82 ± 0.56^{by}	9.73 ± 0.84^{by}	6.85 ± 0.51^{by}
	Diet 3	15.77 ± 0.26^{bx}	17.12 ± 0.37^{cy}	12.52 ± 0.20^{cy}	19.31 ± 0.74^{cx}	10.16 ± 0.59^{bx}	9.81 ± 0.78^{cy}
45 days	Diet 1	12.51 ± 0.50^{ax}	10.37 ± 0.72^{ax}	8.38 ± 0.34^{ax}	14.21 ± 0.49^{ay}	3.63 ± 0.72^{az}	8.08 ± 0.44^{az}
	Diet 2	14.82 ± 0.44^{bz}	15.98 ± 0.42^{by}	9.97 ± 0.15^{bz}	16.53 ± 0.63^{by}	7.35 ± 0.10^{bx}	10.52 ± 0.56^{bx}
	Diet 3	17.46 ± 0.45^{cy}	16.46 ± 0.47^{by}	10.53 ± 0.20^{cx}	19.16 ± 0.67^{cx}	8.58 ± 0.47^{cy}	12.03 ± 0.45^{cx}

[1]Enzyme activity expressed as mean ± s.d. (n=4)

^{abc} Treatment means in each column sharing different superscript for different diets during a particular period differ significantly ($p<0.01$)

^{xyz} Treatment means in each columna sharing different superscript for a particular diet during different periods differ significantly ($p<0.05$)

irrespective of the inclusion level. During the next 15-30 days, it was highest again for the diet containing 5% CS (15.77 μ mol. of pyruvate/ mg protein/ g tissue/ min) and was significantly ($p<0.01$) different from the other two groups. Similarly, during the last 15 days of the experiment, significantly ($p<0.01$) higher enzyme activity of liver (17.46 μ mol. of pyruvate/ mg protein/ g tissue/ min) was observed in the 5% CS group than in the other groups. Livers of fish receiving the diets 1 and 3 showed higher enzymatic activity during the last 15 days than the first 15 days. Enzymatic activity of gill was significantly higher ($p<0.01$) for the fish receiving the diet with 5% CS than for the fish receiving other of gill was lower during the last 15 days than the first 15 days irrespective of the diets. Significantly ($p<0.01$) higher enzymatic activity of muscle was observed for the fish receiving the diet containing cyperus than the fish receiving the control diet for each interval. Supplementation of 5% CS in the diet resulted in highest enzymatic activity of muscle of fish. But irrespective of the diet type, muscle tissue of the fish showed lower enzyme activity during the final 15 days than during the first 15 days.

Ananine aminotransferase

The enzymatic activity of liver was significantly ($p<0.01$) higher in fish receiving the diets supplemented with cyperus than in the control group throughout the experimental period except during the first 15 days. However, highest enzymatic activity of liver was observed with the treatment group receiving 5% CS diet irrespective of the sampling period. Significantly ($p<0.05$) higher enzymatic activity was observed for the fish receiving control diet and diet 2 (1% CS) during the second 15 days than the first 15 days but not during the last 15 days. No significant ($p<0.05$) difference was observed in the enzymatic activity of the liver among the sampling periods for the 5% CS treatment group. The enzymatic activity of gill in fish was significantly ($p<0.01$) higher for the diets supplemented with cyperus than the control diet throughout the experimental period. Highest enzyme activity of gill was observed for the fish receiving diet supplemented with 5% CS in each sampling period. The enzymatic activity of muscle in fish was significantly ($p<0.01$) higher in the cyperus supplemented group than that of control group throughout the experimental period. Highest enzyme activity of muscle was observed for the 5% CS group through out the expermental period.

DISCUSSION

Feeding stimulant supplementation assumes significance in utilization of diets containing plant proteins with low palatability. Takii *et al.* (1986) observed enhanced weight gain and feed efficiency in young eel (*Anguilla japonica*) when fed a diet supplemented with a feeding stimulant mixture (L-alanine, glycine, L-proline, L-histidine and UMP) compared to the control feed in spite of almost the same daily feeding rate between dietary groups. The result suggested that the chemical cues originating from the dietary feeding stimulants might enhance the

cephlic reflex response, resulting in feed intake and weight gain as a result of feeding stimulant supplementation has also been previously observed in gilthead bream (Tandler *et al.,* 1982) and European seabass (Gomes *et al.,* 1997). In the present study, similarly higher growth rate was observed for the fish receiving diets supplemented with cyperus than the control diet. The highest relative growth (72.28%) was obtained with the fish fed with a diet containing 5% cyperus (5% CS) in spite of constant feeding rate (7%) for all the treatment groups. The physiological and nutritional mechanisms underlying the stimulatory effects of dietary feeding stimulants on growth performance of the young fish are not clear. However, the enhanced growth of mrigal reared on the diet with feeding stimulant may be indirectly attributed to increased food intake, more efficient nutrient metabolism, increased digestive and absorptive activities soon after feeding as suggested by Takeda and Takii (1992).

Aminotransferases are intracellular enzymes, which catalyze the transfer of amino group of an amino acid to a keto acid and ultimately synthesize new amino acids (Rodwell, 2000). In this process, nutritionally non-essential amino acids are synthesized within the cell. During enhanced growth, the activity of these enzymes increase to meet the amino acid requirements for protein synthesis and ultimately increases the turn over of amino acids to protein. Two of the hepatic enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) showed higher activities in the feeding stimulant diet group than in the control group (Takii *et al.,* 1986). In the present study, similar results were found. Supplementation of cyperus in the diet irrespective of level resulted in higher AST and ALT activity for liver, gill and muscle than that for the control group. The enhanced growth performance of mrigal may also be attributed to the increased activity of these enzymes related to protein metabolism.

In the context of replacing fishmeal with inexpensive plant proteins for commercial fish culture, use of cyperus as a feeding stimulant assumes significance in improving growth of mrigal. The present study suggests that the cyperus tuber supplementation in the diet improves weight gain in mrigal. Supplementation of cyperus tuber at 5% level in the diet showed better growth performance of mrigal than at 1% level. Under commercial conditions, cyperus powder can be used, in addition to its role as a feeding stimulant, to attract mrigal to certain locations for sampling or harvesting. Further studies are required to identify the components responsible for feeding stimulant activity.

REFERENCES

A.O.A.C. 1990. Official Methods of Analysis. 15th edn. Association of Official Analytical Chemists, Arlington, Virginia.

Carr, W.E.S., Blumenthal, K. M. and Netherton, J. C., 1977. Chemoreception in the pig fish, *Orthopristis chrypterus:* the contribution of amino acid betaine to

stimulation of feeding behaviour by various extracts. *Comp. Biochem. Physio.*, **58A:** 69-73.

Cho, C. Y., Hynes, J. D., Wood, K. R. and Yoshida, H. K., 1991. Quantification of fish culture wastes by biological (nutritional) and chemical (limnological) methods; the development of high-nutrient dense (HND) diets. In: Proceedings, 1st International Symposium of Nutritional Strategies in Management of Aquaculture Waste, University of Guelph, Guelph, Ont., 1990. pp. 37-50.

Food and Agriculture Organization (FAO). 1999. The State of World Fisheries and Aquaculture 1998. Food and Agriculture Organization of United Nations, Rome.

Gomes, E., Dias, J. and Kaushik, S. J., 1997. Improvement of feed intake through supplementation with an attractant mix in European sea bass fed plant protein rich diets. *Aquat. Living Resour.*, **10:** 385-389.

Harada, K., 1990. Attraction activities of spice for oriental weather fish and yellowtail. *Nippon Suisan Gakkaishi.* **56:** 2029-2033.

Harada, K., 1992. Feeding attraction activity of fragrant pungent spice extracts in black abalone, *Haliotis discus.* In: S.A. Shepherd, M. J. Tegner, and S. A. Guzman del Proo (Eds). Abalone of the world. Fishing News Books, Oxford. pp. 193-200.

Ishiwata, N., 1968a. Ecological studies on feeding of fishes - V. Size of fish and satiation amount. *Bull. Jap. Soc. Sci. Fish.*, **34:** 781-784.

Ishiwata, N., 1968b. Ecological studies on feeding of fishes - VI. Factors affecting satiation amount, *Bull. Jap. Soc. Sci. Fish.*, **34:** 785-791.

Johnsen P. B. and Adams, M. S., 1986. Chemical feeding stimulants for the herbivorous fish, *Tilapia zillii. Comp. Biochem. Physiol.*, **83A:** 109-112.

Kohbara, J., Hidaka, I., Morishita, T. and Miyajima, T., 2000. Gustatory and olfactory sensitivity to extracts of jack mackerel muscle in young yellowtail, *Seriola quinqueradiata, Aquaculture.* **181:** 127-140.

Lowry, O. H., Ronebrough, N. J., Farr, A. L. and Randall, R. J., 1951. Protein measurement with Folin Phenol Reagent. *J. Biol. Chem.*, **193:** 265-276.

New, M. B., 1987. Feed and Feedings of Fish and Shrimp manual. FAO and UNEP, Rome. pp. 44-129.

Rodwell, V. W., 2000. Biosynthesis of the Nutritionally Nonessential Amino acids. In: A. B. David, J Ransom and J. Roche (Ed.). Harpers Biochemistry, 25th edition. Appleton and Lange, Norwalk, Connecticut, pp. 307-312.

Tekeda, M. and K. Takii., 1992. Gustation and nutrition in fishes: application to aquaculture. In: T. J. Hara (Ed.) *Fish Chemoreception.* Chapman and Hall, London. pp. 271-287.

Takei, M., 1967. Studies on fish favourite foods *I. Bull. Tokai Reg. Fish. Res. Lab.,* **49:** 119-129.

Takii, K., Shimeno, S. and Takeda, M., 1986. The effect of feeding stimulant in diet on some hepatic enzyme activities of eel. *Bull. Jap. Soc. Sci. Fish.,* **52:** 2131-2134.

Tandler, A., Berg, B. A., Kissil, G. W. and Mackie, A. M., 1982. Effect of food attractants on appetite and Growth rate of gilthead bream, *Sparus aurata L.J. Fish Biol.,* **20:** 673-681.

Wootton, I. D. P., 1964. Microanalysis in medical biochemistry 4 (Ed. J. Churchill and A. Churchill). London pp. 101-107.