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Probiotic Effect of Intestinal Bacteria of Koi Carp *Cyprinus carpio* var. koi

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Two distinct colonies were isolated from the intestine of Koi carp *Cyprinus carpio* var. koi through serial dilution and pour plate method. Based on biochemical tests the isolated colonies were identified as *Lacto bacillus* and Proteus spp. Among the identified microbes mass multiplication of Lacto bacillus in nutrient broth was carried out. Five different feeds having different concentrations of Lacto bacillus such as Feed I (control), Feed II(1 ml), Feed III (2ml), Feed IV (3ml) and Feed V (4ml) were prepared by using fish meal, groundnut oil cake, wheat flour and tapioca flour. Role of microbes on feed utilization parameters were studied for a period of 30 days. The growths, percentage growth, relative growth rate, gross and net growth efficiency were higher in feed II containing 1 ml of microbes. Feed consumption, assimilation and metabolism were higher in control. Feed conversion efficiency was higher in feed III containing 2ml of *Lacto bacillus*. Feed conversion ratio was higher in feed IV.

Key words: Probiotic effect, intestinal bacteria, koi carp.

In ornamental fish culture disease outbreaks are being increasingly recognized as a significant constraint on production and trade, affecting the economic development of the sector in many countries. So far conventional approaches such as the use of disinfectants and antimicrobial drugs, have limited success in the prevention or cure of aquatic diseases, further there is a growing concern about the use and particularly, the abuse of antimicrobial drugs not only in human medicine and agriculture but also in aquaculture.

The massive use of antimicrobials for disease control and growth promotion in animals increases the selective presence exerted on the microbial world and encourages the natural emergence of bacterial resistance. Not only resistant bacteria proliferate after an antibiotic has killed off the other bacteria, but also they can transfer their resistance genes to other bacteria that have never been exposed to the antibiotic. The prophylactic use of antibiotics related to those used in human medicine or the use of any antimicrobial agent known to select for cross resistance to antimicrobials used in human medicine could pose particularly significant hazard to human health. The emphasis in disease management should be

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on prevention which is likely to be more cost effective than cure. This may lead to less reliance on the use of chemicals such as antimicrobials, disinfectants and pesticides, which largely treat the symptoms of the problem and not the cause (Munro *et al* 1995). Recent studies reveal that bacteria present in the intestine of fishes inhibit pathogenic organisms present in the fish and environment. One of the most significant technologies that have evolved in response to disease control problems is the use of probiotics (Browdy, 1990). Probiotics is live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance and that compete the harmful one thus protect the host from diseases. Many researchers have already investigated the relationship of the intestinal micrbiota to the aquatic habitat or food. The bacteria present in the aquatic environment influence the gut micro biota and vice versa. Probiotics may also contribute substantially to the health and zoo technical performance in a nutritional way and although it has sometimes impossible to separate feeding of aquatic organisms from environment. The work related to the isolation of intestinal micro flora of Koi carp and the probiotic effect on growth and survival of Koi carp is totally wanting. Hence the present study was carried out.

MATERIAL AND METHODS

Sample collection

For the present work Koi carp Cyprinus carpio var. koi (2 ± 0.309 g) were collected from Angel Aquarium, Dindigul, Tamil Nadu, India. Intestinal contents from the koi carp were serially diluted with sterile water and plated on nutrient agar. Plates were incubated at 37º C for 24 hrs (pour plate method). After incubation, bacterial colonies were invalid at random from each plate and examined for gram reaction, spore formation, cellular morphology and motility and identified at the genus level. The isolated and identified bacteria Lactobacillus was mass multiplied by inoculating them into the nutrient broth.

Experimental feed preparation

Raw materials are selected based on their ability to supply nutrients such as protein, carbohydrate and fat at low cost. The raw materials used in this study are fish meal, groundnut oil cake, wheat flour, tapioca. After knowing their protein contents by Micro – kjeldhal method (Jeyaraman, 1992) the feeds were prepared (Ali, 1980) along with different levels (1, 2,3 and 4 ml broth) of isolated and identified Lactobacillus.

Methods

Experimental design for growth studies

For the present study Koi carp (2 ± 0.546 g) were collected from Angel Aquarium, Dindigul, Tamil Nadu, India and transported to the laboratory in polythene bags filled with oxygenated water. Fishes were acclimated in plastic round aquaria (60 cm dia) for 15 days. During acclimation, fishes were fed with trainee feed containing fish meal, groundnut oil cake, wheat flour and rice bran in the form of dry pellets. After 15 days uniform size of fish were taken. Then the fishes were introduced in the rectangular glass tanks (45 cm L × 22 cm B

Table 1. Composition of different ingredients used in the experimental feeds (g /100 g)

S.No.	Ingredients	Experimental feeds				
		Feed I(Control)	Feed II	Feed III	Feed IV	Feed V
1.	Fish meal	34.15	34.15	34.15	34.15	34.15
2.	Groundnut oil cake	34.15	34.15	34.15	34.15	34.15
3.	Wheat flour	10.85	10.85	10.85	10.85	10.85
4.	Tapioca	10.85	10.85	10.85	10.85	10.85
5.	Fish oil	2	2	2	2	2
6.	Sunflower oil	2	2	2	2	2
7.	Supplevite – Mix	4	4	4	4	4
8.	Sodium chloride	1	1	1	1	1
9.	Sodium benzoate	1	1	1	1	1
10.	Microbes (cells)	-	1ml	2ml	3ml	4ml

Table 2. Biochemical test

Name of Test	Observation
Gram staining	Gram positive rod
Motility	Motile
Indole	Negative
Methyl red	Negative
Voges proskauer	Negative
Citrate	Negative
Catalase	Negative
Oxidase	Negative

Table 3. Condition Factor (K)

Feeds	Initial	Final
Ex. Feed I (Control)	2.76 ± 0.11	2.61 ± 0.09
Ex. Feed II (1 ml)	2.70 ± 0.14	2.46 ± 0.06
Ex. Feed III (2 ml)	2.77 ± 0.06	2.90 ± 0.04
Ex. Feed IV (3 ml)	2.16 ± 0.23	1.73 ± 0.23
Ex. Feed V (4 ml)	2.51 ± 0.10	2.35 ± 0.07

× 22 cm H) having a capacity of 18 liters. During rearing the fishes were fed on ad – libitum diet of prepared feed twice a day for 1hr each from 9 -10 am and 4 – 5 pm. The unfed were collected after one hour of feeding without disturbing the fishes. The unfed was dried to constant weight. The faecal matter was collected daily before changing the water with least disturbance to the fishes and dried at 95º C. Approximately 70% of water in the tank was replaced with tap water. The experiment was continued for 30 days and on the 30th day the fishes were weighed in live condition. Condition factor (K) was calculated as per Weatherley and Gill (1987) for individual fish before and after the experiment. Feed utilization parameters were calculated.

RESULTS AND DISCUSSION

The organism isolated from the intestinal content was identified as Lacto bacillus spp. using

Table 4. Feed utilization and growth parameters of Koi carp Cyprinus carpio var. koi in relation to different concentrations of Lacto bacillus spp. (cells). Each value is the average (± S.D) performance of 5 individuals in Triplicates reared for 30 days

S. No.	Parameters	Eexperimental feeds				
		Feed I	Feed II	Feed III	Feed IV	Feed V
1.	Feed Consumption(FC) (g/g live wt/ 30 days)	4.99 ± 0.41a	4.49 ± 0.01b	4.48 ± 0.01c	4.55 ± 0.02d	4.77 ± 0.03e
2.	Feed Conversion Efficiency (FCE)	0.11 ± 0.01	0.13 ± 0.03	0.14 ± 0.01	0.12 ± 0.03	0.10 ± 0.01
3.	Feed Conversion Ratio (FCR)	10.04 ± 0.10	7.51 ± 1.21	7.67 ± 0.12	9.96 ± 0.11	10.63 ± 1.73
4.	Growth (G) (g/g live wt/ 30 days)	0.49 ± 0.02a	0.63 ± 0.23b	0.58 ± 0.01c	0.46± 0.01d	0.47 ± 0.07e
5.	Percentage Growth (PG) (%)	24.12 ± 0.36	31.44 ± 6.03	28.92 ± 0.32	22.3 ± 0.8	22.3 ± 0.65
6.	Relative Growth Rate (RGR)	0.24 ± 0.01	0.31 ± 0.03	0.29 ± 0.01	0.22 ± 0.01	0.25 ± 0.03
7.	Assimilation (A)	2.68 ± 0.14	2.24 ± 0.12	2.41 ± 0.05	2.22 ± 0.01	2.25 ± 0.03
8.	Metabolism (M)	2.19 ± 0.09	1.61 ± 0.16	1.82 ± 0.04	1.77 ± 0.08	1.77 ± 0.22
9.	Gross Growth Efficiency (GGE) (%)	9.92 ± 0.12a	14.01 ± 2.19b	13.02 ± 0.20c	10.02 ± 0.12d	9.88 ± 1.51e
10.	Net Growth Efficiency (NGE) (%)	18.49 ± 0.80a	28.34 ± 5.19b	24.21 ± 0.50c	20.57 ± 1.17d	19.2 ± 1.05e

Feed Consumption	Growth	Gross Growth Efficiency	Net Growth Efficiency
a vs b (P > 0.05) NS	a vs b (P > 0.05) S	a vs b (P > 0.05) S	a vs b (P > 0.05) S
a vs c (P > 0.05) NS	a vs c (P > 0.05) S	a vs c (P > 0.05) S	a vs b (P > 0.05) S
a vs d (P > 0.05) NS	a vs d (P > 0.05) NS	a vs d (P > 0.05) S	a vs b (P > 0.05) S
a vs e (P > 0.05) NS	a vs e (P > 0.05) NS	a vs e (P > 0.05) NS	a vs b (P > 0.05) S

biochemical tests (Table 2).The fresh water fishes harbour human pathogenic bacteria including Aeromonas spp. and Staphylococcus faecalis, Salmonella sp. and Staphylococcus spp. in their intestine. Many workers have isolated bacteria from marine sources, fish with probiotic effect of lactic acid bacteria in the feed on growth and survival of fry of Atlantic cod (Gildberg et al 1995). Condition factor (K) of Koi carp was estimated for comparative purposes to assess the feed. The average initial condition factor is 2.58 and the final condition factor increased in feed III (2.90) and in all others the condition factor is decreased. Feed consumption of Koi carp was higher in feed V containing 4 ml of Lacto bacillus spp. The different feed utilization and growth parameters are presented in Table 3.The feed consumption in feed I is not significantly varied with other experimental feeds. Jeyachristina Arockia Selvi (2005) reported that the feed consumption of Gold fish was higher in feed V containing higher cells of Pseudomonas spp. Feed conversion efficiency was higher in feed III containing 2 ml of Lacto bacillus spp. In feed IV and V The feed conversion efficiency were gradually decreased .But in the case of Gold fish, the feed conversion efficiency gradually decreased from lower to higher concentration of Pseudomonas spp. Feed conversion ratio was best in feed V containing 4 ml of Lacto bacillus spp. In feed I, feed conversion ratio was decreased. Same result was reported in gold fish (Jeyachristina Arockia Selvi, 2005).Growth, Percentage growth and Relative growth rate was higher in feed II containing 1 ml of Lacto bacillus. Assimilation, metabolism, gross and net growth efficiency of Koi carp was higher in feed II. From the results , it is inferred that some of the feed utilization parameters such as Growth, Percentage growth, Relative growth rate, Gross and Net growth efficiency were higher in feed II containing lower cells of Lacto bacillus spp.

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REFERENCES

1. Munro, P. D., A. Barbour and T.H.Birkbeck. Comparison of the growth and survival of larval turbot in the absence of culturable bacteria with those in the presence of *Vibrio anguillarum, Vibrio alginolyticus* and a marine *Aeromonas* spp. *Appl. Environ. Microbiol***,** 1995; **61**: 4425 – 4428.
2. Browdy, C. Recent development in penaeid brood stock and seed production technologies : Improving the outlook for superior captive stocks. *Aquaculture,* 1990. **164:** 3-21.
3. Jeyaraman, J. Laboratory Manuel in biochemistry. Wiley Eastern Ltd, New Delhi, Fourth reprint, 1992; 75-78.
4. Ali, S. A. Feed formulation method. Manuel of research methods for fin fish and shell fish nutrition. CMFRI Special publication, 1980. **8**: 98.
5. Weatherley, A. H. and H.S. Gill. The biology of fish growth. Academic press, London, UK.1987.