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Influence of Probiotics on the Growth and Gut Microbial Load of Juvenile Goldfish (*Carassius auratus*)

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Abstract

The investigation was aimed at determining the influence of probiotics on the growth and gut microbial load of juvenile goldfish. Three probiotics viz., sporolac, lactobacil and yeast were added to the basal diets at 0.5% level and the juvenile goldfish were fed for a period of 60 days. Sporolac incorporated feed recorded a maximum mean weight gain of 0.673 g and a six-fold increase in growth compared to control feed. The percentage composition of *Lactobacillus* in the *Carassius auratus* fed with control feed was comparatively low (2.7) than those supplemented with probiotics.

Introduction

Aquarium fishes are rapidly gaining importance not only because of their aesthetic value but also due to their immense commercial value in the export trade the world over. So many feed additives have been tried to improve the feed utilization efficiency in finfish and shellfish. In the early 1950's, antibiotics were used as common feed additive. The extensive and continuous usage of antibiotics is known to help the production of antibiotic resistant bacteria which may directly affect the genetic material causing mutation. In order to overcome all these problems, scientists have selected certain beneficial microbes, to be used as feed additives. Parker (1974) coined the term probiotic and defined the term as "organisms and substances which contribute to intestinal microbial balance". Probiotics can also be considered as microbes to improve the nutritive value of an animal feed (Castellanos et

al. 1996). Several studies have shown that probiotics can improve the growth rate of the fishes. (Noh et al. 1994, Metaillier and Hollocou 1993, Gildberg et al. 1995, Queiroz and Boyd 1998 and Gatesoupe 1991). The present investigation was conducted to study the influence of probiotics on the growth and gut microbial load of juvenile goldfish.

Materials and Methods

Healthy goldfish weighing from 0.257 to 0.326 g (25 to 32 mm) taken from the acclimatization tank were stocked in experimental plastic tubs of 47 cm dia and 13 cm water depth. The fishes were stocked in all the twelve tubs (triplicates for each treatment) at the rate of 10 per tub. Different ingredients were procured from local markets in and around Thoothukkudi.(Table 1) All the ingredients (except lactobacil, sporolac, and yeast) were weighed, powdered and mixed thoroughly using a mixer grinder and passed through 250 µ seive for uniformity. The ingredients were mixed well with water and cooked in a pressure cooker for 30 min. The cooled paste was divided into four equal parts by weight. One part was kept as control feed. The other parts were used for mixing the probiotics and used as test diets. The feed was made into powdered form and fed to the juvenile fish at the rate of 10% of their body weight.

Physico-chemical parameters such as temperature, pH, dissolved oxygen, ammonia, nitrite and nitrate were estimated following standard procedures. The experiment was conducted for a period of 60 days. The sampling was carried out once in a fortnight.

The bacteriological analysis of the gut flora of the fishes was carried out after the completion of the experiment. The wet weight of each fish was determined before the dissection. The fishes were dissected to remove the intestine, after washing the ventral surface area of the fish with sterile distilled water. The entire intestine was homogenized in 9 ml of the diluents (Phosphate buffer saline, 0.01 molar) and mixed with a vortex mixer and used as 10^{-1} dilution. Each sample was serially diluted, using pour plate method, poured into four different media. The total plate count (TPC) was

Table 1. Ingredients used in feed preparation

Sl. No.	Ingredients	Purpose of Inclusion	Inclusion level %
1.	Fish meal	Animal protein	16%
2.	Groundnut oil cake	Plant protein	16%
3.	Sesame oil cake	Plant protein	16%
4.	Soya flour	Plant protein	16%
5.	Rice bran	Carbohydrate	18%
6.	Tapioca flour	Binder	18%
7.	Vitamins and mineral mix	Vitamin & minerals	0.5%
8.	Lactobacil UNI-Sankyo Ltd, Ratnagiri, Maharashtra.	Probiotic	0.25% (2.5 g•kg)
9.	Sporolac Inter Care Ltd. Mehsana, Gujarat.	Probiotic	0.25% (2.5 g•kg)
10.	YeastLocal Bakery, Tuticorin.	Probiotic	0.25% (2.5 g•kg)

determined using Tryptone soya Agar (TSA) media. The lactobacilli count was determined using MRS agar (De Man, Rogosa and Sharpe 1960) media. The yeast count was determined using YDP agar (Kreger – Van Rij 1984) media. The inoculated plates were incubated at 28 ± 2°C for 3 days. The anaerobic count was determined using anaerobic agar media. The inoculated plates were incubated at 28 ± 2°C for 3 days in anaerobic condition. The total coliform count was determined using the most probable number method (MPN).

Bacterial cultures were isolated from TSA, MRS and YDP plates. The isolated cultures were purified by repeated streaking on TSA plates and these were maintained on TSA slants for morphological and biochemical characterization. Morphology of the bacterial cultures was observed in 16-24 hr old cultures grown on TSA plates after gram staining. Presence of spores was detected in 48-72 h old bacterial cultures after staining and examination through microscope. Motility of the culture was observed through the hanging drop method (Koneman et al. 1988)

The biochemical reactions of the cultures were determined by standard methods (Salle 1954; FDA 1973), the mode of attack of glucose by bacterial culture was determined using the Hi media of Hugh and Leifson (1953). The presence of cytochrome oxidase in the cultures was detected by the modified Kovacs test. The catalase test was done by observing the evolution of gas when the 1/10 dilution H_2O_2 was mixed with a speck of young culture on clean slide and sensitivity to penicillin (2.51 µ) by the pad plate method. The differentiation of the bacterial cultures up to the generic level was done following the scheme of Surendran (1980).

Results

The data regarding the growth of juvenile *C. auratus* tested with three different probiotic incorporated feeds are presented in table 2. The mean weight gain at the end of the experiment for the sporolac and lactobacil treatment was 0.673 and 0.328 g respectively. The mean weight gain in the fishes fed with yeast was recorded to be 0.344 g. A mean weight increase of 0.112 g was observed in the fishes fed with control feed. A maximum weight gain of 0.673 g was recorded in the fishes fed with sporolac followed by

Table 2. Effect of three different probiotics on the growth of juvenile *C. auratus* (P- 0.036)

Sl. No.	Treatment	Mean weight (g)					Mean weight gain (g)	Specific growth rate	t -value	
		1st day	15th day	30th day	45th day	60th day				
1.	Sporolac	0.326	0.361	0.391	0.506	0.999	0.673	2.22	2.10	(P>0.05)
2.	Lactobacil	0.271	0.327	0.403	0.511	0.599	0.328	0.85	3.12	(P<0.05)
3.	Yeast	0.301	0.323	0.370	0.469	0.645	0.344	0.93	2.95	(P<0.05)
4.	Control	0.257	0.262	0.283	0.313	0.369	0.112	0.26	-	-

yeast (0.344 g) and lactobacil (0.328 g). The specific growth rate of the fishes was also determined.

Water quality parameters

The water temperature of the experimental tubs varied from 26 to 27.5°C at 8 am and 27 to 28°C at 5 pm.The water pH was found to fluctuate between 7.84 and 8.66.The dissolved oxygen content showed variation from 6.56 to 8.96 mg•l at 8 am and 7.8 to 12.3 mg•l at 5 pm. The nitrite level of 0.05 to 0.25 mg•l was measured in experimental tubs. Nitrate level showed variation from 0 to 0.25 mg•l. The presence of ammonia was in trace level throughout the experimental period. The proximate composition of the four experimental feeds is presented in table 3.

The total viable counts (cells•g) observed in the gut of juvenile goldfish are presented in table 4. *Lactobacillus* sp. was presumptively identified by the following test results:

1. Gram's staining — Gram positive (violet color)
2. Colony morphology — Small rods, coccobacilli
3. Motility — Non motile
4. Catalase — Negative
5. Spore staining — No spore formation

The yeast cells were presumptively identified with the following tests:

1. Gram's staining — Gram positive (violet color)
2. Colony morphology — Large round cells

Table 3. Proximate composition of experimental feeds

Feed	Protein (%)	Fat (%)	Ash (%)	Moisture (%)
Sporolac	26.5	4.50	9.80	6.31
Lactobacil	26.0	4.65	9.90	6.40
Yeast	26.0	4.27	9.87	6.60
Control	26.0	4.30	9.90	6.33

Table 4. Viable counts (cells•g) of different bacterial components in the gut flora of juvenile *C. auratus*

Sl. No.	Viable count	Treatments			
		Sporolac	Lactobacil	Yeast	Control
1.	Total plate count	$8x10^8$	$2.5x10^8$	$7x10^7$	$5.7x10^8$
2.	Total coliforms	1300	1350	1110	1583
3.	Total fecal coliforms	300	150	166	333
4.	*Lacto bacillus*	$4.8x10^7$	$6.0x10^7$	-	$1.6x10^6$
5.	Yeast	-	-	$2.6x10^6$	-
6.	Facultative anaerobic count	$8.1x10^7$	$3.2x10^6$	$7.1x10^6$	$6.8x10^7$

Qualitative analysis of microbes in the gut of juvenile C. auratus

A total of 12 bacterial strains were isolated and presumptively identified up to the generic level and their percentage composition was also calculated and presented in table 5. The Micrococcaceae formed 3.3, 16.7, 20 and 20% of the total microbes in the sporolac, lactobacil, yeast and control fishes respectively. The *Lactobacillus* percentage in the gut was recorded as 10, 10, 6.7 and 2.7% in the sporolac, lactobacil, yeast and control respectively. The percentage composition of *Bacillus* was 6.7, 3.3, 13.3 and 26.7% while the *Vibrio* percentage stood at 16.7, 16.7, 10 and 13.3% in the sporolac, lactobacil, yeast and control fishes respectively.

Discussion

The present work was carried out to determine the influence of probiotics on the growth and gut microbial load of juvenile goldfish. The mean weight gain on the 60th day of the experiment was found to be maximum in *C. auratus* fed with sporolac. It recorded a mean weight gain of 0.673 g. The specific growth rate of the fish was also found to be maximum (2.22) in the fishes fed with sporolac incorporated diet. The fishes fed with yeast incorporated feed showed a mean weight gain of 0.344 g which was closely followed by lactobacil (0.328 g). A mean weight gain of 0.112 g was observed in the fishes fed with control feed. The growth of juvenile *C. auratus* was more or less the same in lactobacil and yeast incorporated feed. A significant difference in growth was observed between the probiotic treated feeds and control feed. A six-fold increase in growth was observed in the sporolac treatment compared to the control and a 3-fold increase in

Table 5. Percentage of microorganisms in the gut of juvenile *Carassius auratus* fed with different probiotics

Sl. No.	Microorganisms*	Treatments			
		Sporolac (%)	Lactobacil (%)	Yeast (%)	Control (%)
1.	Micrococcaceae	3.3	16.7	20	20
2.	*Arthrobacter*	30	40	20	3.3
3.	*Lactobacillus*	10	10	6.7	2.7
4.	*Bacillus*	6.7	3.3	13.3	26.7
5.	Vibrio	16.7	16.7	10	13.3
6.	*Acinetobacter*	-	-	-	3.3
7.	Entrobacteriaceae	-	3.3	-	3.3
8.	*Moraxella*	3.3	-	-	-
9.	*Alcalgenes*	-	-	-	-
10.	Uncharacterised	30	6.7	30	43.3

*Surendran, 1980

growth was observed in the case of lactobacil and yeast treated feeds. The probiotics used as growth promoters in *C. auratus* in the experiment showed significant growth in the juvenile goldfish. Similar observations were also made by Noh et al. (1994) in carps, Metaillier and Hollocou (1993) in Atlantic salmon and Gildberg et al. (1995 1997) in Atlantic cod.

Queiroz and Boyd (1998) and Gatesoupe (1991) also stated that the probiotic incorporated feed had a definite role in enhancing the growth of channel catfish and turbot larvae. The present study also confirmed the findings of Ringpipat, et al. (1998), Mae da and Liao. (1992) and Garriques and Arevalo (1995) who reported a significant increment in growth of *Penaeus monodon* and *Penaeus vannamei* fed with probiotic incorporated feeds.

The gut flora of the juvenile goldfish was analysed at the end of the experiment. In the gut, the *Lactobacillus* count was observed to be 4.8×10^7 and 6.0×10^7 cells•g. in sporolac and lactobacil treated fishes respectively. The presence of *Lactobacillus* was also observed in the control (1.6×10^4 cells•g). The total count of yeast was found as 2.6×10^6 cells•g in the gut of fishes fed with yeast incorporated feed. The percentage of yeast was not observed in the treatments and control. The coliforms and fecal coliforms were observed in the gut of all experimental fishes but *E.coli* was completely absent in the gut.

In the qualitative analysis of gut flora of juvenile fish the presumptively identified microbes were Micrococcaceae, *Arthrobacter, Lactobacillus, Bacillus, Vibrio, Pseudomonas, Acinetobacter,* Entrobacteriaceae and *Alcalgenes.* In this study the percentage values of *Lactobacillus* found in the gut of fishes were 10, 10, 6.7 and 2.7 % in sporolac, lactobacil, yeast and control fishes respectively. Ringo (1993) reported that the lactic acid bacteria were part of the normal microflora of the gastro intestinal tract of both hatchery cultured and wild caught Arctic charr, (*Salvelinus alpinus* L.). The present results also confirmed the results of Sugita et al. (1988) who reported more or less similar types of microbes in the intestine of juvenile goldfish.

The percentage composition of *Lactobacillus* in the *C. auratus* fed with control feed was comparatively lower (2.7) than those supplemented with probiotics. These results confirmed the findings of Gildberg et al. (1995; 1997) who observed that the lactic acid bacteria are rarely present in the juvenile fish reared on artificial diets. However such bacteria could become dominant in the intestinal flora only when they are supplemented in the feed. Ringo and Gatesoupe (1998) reported that several species of *Lactobacillus* form part of the natural intestinal flora of healthy fish.

Arthrobacter and *Vibrio* load was high in the gut of juvenile fish when compared to other components. The level of uncharacterized microbes observed in the present study was 30, 6.7, 30 and 43.3% in the sporolac, lactobacil, yeast and control fishes respectively. An extremely higher percentage of uncharacterized microbes found in the fishes fed with control feed suggested that the probiotics in the gut of the fishes suppressed the growth of other microbes, (Ringo et al. 1995 and Montes and Pugh 1993).

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