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Research Article

Effect of probiotic *Spirulina fusiformis* feed on growth performance of *Catla catla*

Venkatramalingam K and Saravanan N*

Research Department of Zoology, Government Arts College (A), Salem, Tamil Nadu

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***CORRESPONDENCE**
drnsaran@yahoo.com

ABSTRACT

Probiotics are functional ingredients and their incorporation in aqua feeds was considered a possible strategy to enhance the growth of fish. Spirulina acts as a growth promoter, probiotic food booster of the immune system in animals including fishes. Spirulina is truly an amazing food full of nutritional wonders. Spirulina species are most commonly used in nutritional supplements. It is cheaper feed ingredient than other animal origin. A 60 days experiment was done with a view to analyze the effects of probiotic Spirulina diets on the growth of *Catla catla* fingerlings. Results proved that the Spirulina feeds elicited the beneficial effect and it can increase the survivability of fish and accelerates the growth of *C. catla* fingerlings. Fish fed with *Spirulina fusiformis* (5%) had maximum feed consumption (20.31g). The maximum feeding rate of 366.58 mg was determined in fish consuming 5% spirulina. The maximum weight of 6.22g was noted in SF5 feed (5%). However the minimum increase in bodyweight was 2.60 and 3.98g in control and 1% inclusion of spirulina feed. The highest enhancement in length (4.53cm) was observed in fishes fed with SF5. In the present study spirulina found to have a potential effect on SGR, it is higher (0.68 %) in SF5 spirulina diet. Food conversion ratio (FCR) was observed from 3.07 to 3.93 in fishes fed with the diet containing spirulina. The present study showed a linear relationship existed between dietary supplementation of *S. fusiformis* level and FCR.

INTRODUCTION

Fisheries have always play a very significant social economic role in many countries. India is the second-largest aquaculture producer in the World, about 80% of India's aquaculture production is dominated by carp production. Feed is one of the important inputs in aquaculture, and the success of aqua farming depends mainly on the adequate quantity of nutritionally balanced feed. Supplementary feeding is known to promote the carrying capacity of culture systems and can improve fish production by many folds. Different feed additives such as probiotics and single cell protein may also be used to exploit the maximum growth potential of animals (Ahmad *et al*., 1995; Salminen *et al*., 1999). In fish farming, nutrition is important because feed represents 40-50% of the production costs. Fish nutrition has upgraded dramatically in recent years with the development of new, balanced supplementary diets that enhance optimal fish growth and health (Mohapatra *et al*., 2013).

Aquaculture development has become a great task for future generations, not only for feed cost but also the availability of fish meal and ingredients. Consequently, alternative feed ingredients development is urgent and they must meet the animal nutritional requirements, be less and sustainable with the environment. The utilization of selected probiotic organisms has emerged as a solution with huge application in the aquaculture feed industry .The probiotic feed inhibits pathogen proliferations, increases health status of fish ,improving food conversion ratio and indices optimal growth of fishes(Olmos *et al*., 2015).A probiotic may also be a functional feed and are defined as; food that contain some health promoting components beyond traditional nutrients. Recent years, new food products have been formulated with the addition of probiotic organisms (Scheinbach, 1998). Feeding on spirulina helped to improve disease resistance of fish resulting in an improvement in their survival rate (Ghaeni *et al*., 2011).

In aquaculture, probiotic feeds are believed to provide various health benefits, like supply of nutrients, enzymes, enhancement of specific and nonspecific immunity, prevention of intestinal disorders, diseases control and water quality maintenance to attain improvement in survival, feed efficiency, growth and production (Susmita *et al.*, 2017). Spirulina can partially replace fishmeal proteins in fish feeds; it enhances growth in the fish. Probiotics are live microbial cells that are administered to gastrointestinal tract of the host as a feed supplement. Better growth rates are obtained, and less feed is wasted because of inherent palatability of spirulina.

Spirulina feeds are rich in nutrients, with the high protein content, vitamins, essential amino acids and fatty acids (Kim *et al*., 2013).In addition to high quality proteins, it contains high amounts of calcium, vitamin B12 ,enzymes and also very rich in iron content. Spirulina feed has been used as a nutrient for fish larvae and ingredient in fish diet for juveniles and adult common carps (Palmegiano *et al*., 2008).Several studies have been conducted using dried spirulina as a feed supplement for fishes (Mukherjee *et al*., 2011; Roy *et al*., 2011).

S. fusiformis holds potential for inclusion in diets of various fish species due to its attractive nutrient profile and digestibility. Spirulina supplemented diet showed a marked development in color, texture and taste. Spirulina could be an excellent source of useful nutrients as well as a good energy source that can be used as crucial component of animals feeding (Kim *et al.*, 2013). Spirulina can be used as a partial supplementation or complete replacement for protein in aqua feeds and is cheaper feed ingredient than other animal origin (Rosas *et al.*, 2019).The success of Spirulina diet replacement would save feed costs, improve the growth performance, feed conversion ratio and survival rate. Keeping this in view, the present study has been carried out to find the effect of probiotic spirulina incorporated diet in enhancing the growth performance and feed utilization in the fingerlings of *Catla catla.*

MATERIALS AND METHODS

Collection of fishes

The fingerlings of *C. catla* were procured from fish farm, in Mettur, Salem district. They were carefully transported to the laboratory through oxygenated polythene bags. These animals were brought to the lab condition and were stocked in the plastic troughs. These fishes were allowed to acclimatize for a period of 10 days. Then they were subjected to experiments.

Preparation of probiotic diets

The experimental diets were prepared with selective feed ingredients viz; corn flour, wheat flour, rice bran, groundnut oil cake, tapioca powder, Agar agar, dried spent silk moth power, cod liver oil, vitamins and minerals as per 'pearson square method' using predetermined values of 40% protein (Table-1). The experimental probiotic Spirulina feed were prepared with pure culture isolates of *Spirulina fusiformis* at various proportions (1, 2, 3, 4 & 5%) respectively. The feed without spirulina is used as control feed. The feed included with spirulina are named as SF1, SF2, SF3, SF4, and SF5 (Table-1).

Experimental set up and feeding

Experiment was conducted over two months (60 days) period at laboratory. Fingerlings having same size, their average live body weight was 3.00±0.05g and the average total length was 4.00±1.00 cm were selected. Their sex was not taken into consideration. Feeding experiments were conducted with control diet and experimental feeds. Experimental fishes were placed in a trough with a size of 40 x 15cms, which is filled with 20 L of water. Each feed treatment was applied in triplicates. Feeding rate for all the diet types were 10% of body biomass. The fish were fed once in the morning. Satiations were determined based on visual observation of acceptance and refusal of feed. The unconsumed feed and excrement from the bottom of the troughs were siphoned with a rubber pipe daily. The dissolved oxygen level of water was maintained at 5.10 ± 1 mg/lt. To supply oxygen to the troughs, aerators were used.

After two weeks, cultured experiment fish fingerlings were captured from each treatment using drag net and weighed. All fish weights were taken in an electrical digital balance to an accuracy of 1mg. After recording the wet body weight, they were released back into their respective troughs. The total length of fish was also measured at weekly intervals. The feeding regime (10%) was readjusted at every two week intervals on the basis of wet weight gain . At the end of the experiment (60 days), fish fingerlings from all treatments were weighed, based on which the growth parameters like weight gain (%), Feed Conversion Ratio (FCR), Specific Growth Rate (SGR) and Survival (%) were calculated.

Bio energetic studies

Food utilization

The scheme of energy budget was calculated using IBP formula of Petrusewicz and Macfadyen (1970) represented as C = P + R + F + U, Where C = Food consumption, P = Production (growth), R = Respiration (metabolism), F = Feces and U = Nitrogenous excretory products (urine)

Food consumption (C)

Food consumption was estimated gravimetrically in terms of dry weight by subtracting the dry weight of the unfed from the dry weight of the food supplied.
Feed consumed = Total feed consumed (mg/g) – Feed unconsumed (mg/g)

Food absorbed (A)

Food absorbed was estimated by subtracting the dry weight of feces from that of food consumed. (A = C – F).

Food converted (P)

Food converted was calculated by subtracting the dry weight of fish at the commencement of the experiment from the dry weight of the fish at the termination of the experiment. To study their initial dry weight, control samples consisting two testing individuals of *C. catla* were separately weighed and dried at 60^0C.

Food metabolism (R)

Total metabolism was determined as the difference between absorption and growth (R=A-P)

Food metabolized = Food absorbed – Wet weight gain of fish

Energy budget and growth indices

Rates of feeding, absorption, conversion and metabolism were calculated by dividing the respective quantities with initial weight of the fish (g) and duration of the experiment (Sambath and Lily Premila, 1995). The rate was expressed as mg dry wt/gm live fish / day.

Statistical methods

Data obtained were subjected to the analysis of variance (ANOVA) and correlation analysis. SPSS (V 20.0) was applied to determine whether significant variations between control and experiment values. Difference between means were determined and compared by Duncan's multiple range test (DMRT) and the significances are mentioned. The data are represented as mean $\pm$ standard deviation.

RESULTS AND DISCUSSION

The experiment was carried out for 60 days with a view to analyze the effects of probiotic Spirulina diet on the growth of *C. catla* fingerlings. At the end of the feeding experiments, the growth parameters such as wet weight gain, specific growth rate, food conversion ratio, fish length gain; food consumption rate, metabolic rate, food conversion efficiency and bioenergetics were calculated. Results proved that the Spirulina feed elicited the beneficial effect and it can increase the survivability of fish and accelerates the growth of *C. catla* fingerlings. The feed inputs, recovery of the fecal matter and unconsumed feeds from each treatment were calculated. Three fishes from each treatment spirulina feed were sampled at 15 days intervals and accordingly the amount of feed given was altered.

The results showed that different experiments (SF1, SF2, SF3, SF4, SF5%) had significant impact on the feeding parameters. The quantity of feed consumption of fish fingerlings were varied, fish fed with spirulina (5%) had maximum feed consumption (20.31g) than other experimental feeds. The minimum consumption (12.00 and 15.07g) was noted in control diet and SF1 diet respectively (Table-2).In this study the experimental diet fed group manifested a peak level of consumption. Regarding the absorption, the highest value of 18.00g was noticed in SF5feed (5%) diet and the lowest 12.00 g was recorded in control diet feed type. The food metabolized were higher (19.92g) in SF5 (5%) and lower (10.00g) in control feed.

The maximum feeding rate of 366.58 mg was determined in fish consuming 5% spirulina (SF5) where as the minimum feeding rate of 268.00mg was monitored in control feed (Table-2). The absorption rate was found to be higher (324.00 mg) and lower (230.00 mg) in 5 % spirulina feed (SF5) and control feed respectively. 5 % spirulina exhibited significant absorption rate. The highest food absorption efficiency was found to be 92.11 % in SF5 feed. The highest food gross conversion efficiency was found to be high (19.52%) in SF4, which is significantly higher than the rest of the treatments. The net conversion efficiency was ranged from 14.47 to 20.81%.The maximum net conversion efficiency (20.81%) and minimum net conversion efficiency (14.47%) was noticed in SF5 and control feed respectively (Table-2).

The results of the current study showed that growth performance (LG, WG, FDG and SGR) increased with increasing *S. fusiformis* inclusion. This increase could possibly be due to the improved feed intake and nutrient digestibility. The growth rate of fish fingerlings fed with different feed type is depicted in Table-3. Replacing spirulina increased carcass mean weight compared to other treatments. Diet administered to fingerlings, supplemented with spirulina produced best growth rate 3.98, 4.36, 5.48, 6.22 and 5.13g in SF1, SF2, SF3, SF4, and SF5 feed respectively. The maximum weight of 6.22g was noted in SF5 feed (5%). However a minimum increase in body weight (2.60g and 3.98g) was also observed in control and 1% of spirulina feed.

A significant difference in length gain was found between treatment groups compared to control group, where the highest enhancement in length (4.53cm) was observed in fishes fed with SF5 (5%) diet and minimum (2.28cm) in control diet .The superior range (194.98%) of percent weight gain and length gain (106.34%) was observed in SF5 and SF3 probiotic *S. fusiformis* feed. Under natural and experimental conditions, the status of the fish health can be measured by its specific growth rate. In the present study spirulina has potential effect on SGR, it is higher (0.68 %) in SF5 diet and lower (0.46%) in control feed. Food conversion ratio (FCR) was observed that fishes fed with the diet containing spirulina showed (3.07 to 3.93) values. Food conversion ratio (FCR) was observed to be highest (3.93) in SF5 spirulina feed and lowest (3.07) was noticed in control feed type (Table-3). The present study has showed a linear relationship existed between dietary supplementation of *S. fusiformis* level and FCR.

Table 1 Composition of probiotic *S. fusiformis* incorporated experimental fish feeds.

Sl. No	Ingredients	Feed types and quantity of each ingredient (g/100g)					
		Control (0%)	SF1 (1%)	SF2 (2%)	SF3 (3%)	SF4 (4%)	SF5 (5%)
1	Dried silk moth powder	23.13	22.89	22.89	22.89	22.89	22.89
2	Groundnut oil cake	16.15	15.51	15.51	15.51	15.51	14.51
3	Rice bran	11.75	11.63	11.63	10.63	10.63	10.63
4	Corn flour	17.35	17.17	16.17	16.17	15.17	15.17
5	Wheat flour	17.35	17.17	17.17	17.17	17.17	17.17
6	Tapioca powder	10.27	10.63	10.63	10.63	10.63	10.63

7	Cod liver oil	2.00	2.00	2.00	2.00	2.00	2.00
8	Vitamins & Minerals	1.00	1.00	1.00	1.00	1.00	1.00
9	Agar	1.00	1.00	1.00	1.00	1.00	1.00
10	*S. fusiformis* powder	_	1.00	2.00	3 .00	4.00	5.00

Table 2 Bioenergetics of *C. catla* fingerlings fed with different percentage of probiotic spirulina feed

Parameters	Experimental feed types					
	Control	**SF1**	**SF2**	**SF3**	**SF4**	**SF5**
Consumption*	12.00±0.46	15.07±0.63[a]	17.00±0.12[a]	18.10.±0.10[d]	19.71±0.28[d]	20.31± 0.47[d]
Absorption*	12.00±0.47	14.60±0.83[a]	15.20±0.15[a]	17.00±0.29[d]	17.50 ±0.34[d]	18.00±0.14[d]
Metabolized*	10.00±0.10	11.06±0.67[a]	13.343±0.30[b]	15.405±0.17[d]	18.00 ± 0.32[d]	19.92±0.31[b]
Feeding rate*	268.00±4.75	300.76±10.47[d]	318.04±6.71[b]	330.90±5.13[b]	358.96±9.63[c]	366.58±21.11[c]
Absorption rate*	230.14±2.00	260.03±5.65[b]	290.10±8.00[b]	280.00±7.02[b]	324.00±10.40[b]	307.35±10.01[d]
Metabolized rate*	200.00±2.18	220.00±10.78[d]	230.00±6.18[d]	239.30±4.14[a]	263.20±10.00[b]	251.10±11.10[b]
Absorption efficiency**	85.71±1.99	85.15±0.52[d]	85.38±1.10[d]	90.90±2.17[d]	90.28±0.95[a]	92.11±5.19[d]
Gross conversion efficiency**	12.59±1.10	13.38±1.29[d]	16.48±0.32[d]	15.23±0.41[d]	18.70±0.48[a]	19.52±0.19[a]
Net conversion efficiency**	14.47±0.86	15.20±1.38[d]	17.02±0.11[d]	17.27±0.59[d]	20.81±0.43[b]	20.00±0.66[b]

**g.dry.wt./wt.of.fish/days; ** Percent (%)*
Each value is mean ± standard deviation of triplicate observation.
Mean values within the same row sharing different superscript are significantly different (P<0.05).
Value within the same column sharing the same superscript is not significantly different (P<0.05).

Table 3 Growth performances and food conversion ratio of *C. catla* fingerlings fed with different percentage of probiotic spirulina feeds.

Parameters	Feed types					
	Control	**SF1**	**SF2**	**SF3**	**SF4**	**SF5**
Initial weight(g)	3.00 ± 0.17	3.02 ± 0.06	3.03 ± 0.02	3.10 ± 0.04	3.10 ± 0.02	3.19 ± 0.04
Final weight(g)	5.60 ± 0.10	7.00 ± 0.09	7.39 ± 0.33	8.58 ± 0.51	8.23 ± 0.21	9.41 ± 0.29
Net weight gain(g)	2.60 ± 0.09	3.98 ± 0.08	4.36 ± 0.05	5.48 ± 0.33	5.13 ± 0.08	6.22 ± 0.33
Initial length(mm)	4.70 ± 0.15	4.90 ± 0.10	4.60 ± 0.08	4.10 ± 0.20	4.41 ± 0.09	4.40 ± 0.10
Final length(mm)	6.98 ± 0.09	7.53 ± 0.25	8.00 ± 0.09	8.46 ± 0.12	8.04 ± 0.13	8.93 ± 0.17
Net length gain(mm)	2.28 ± 0.16	2.63 ± 0.09	3.40 ± 0.10	4.36 ± 0.15	3.63 ± 0.14	4.53 ± 0.16
Percent gain in weight (%)	86.67 ± 3.57	131.78 ± 2.33	143.88 ± 5.60	176.77 ± 12.92	165.48 ± 2.67	194.98 ± 4.00
Percent gain in length (%)	48.51 ± 3.07	53.67 ± 1.00	73.91 ± 2.58	106.34 ± 4.90	82.32 ± 3.34	102.95 ± 2.18
Specific growth rate (SGR)	0.46 ± 0.01	0.53 ± 0.02	0.64 ± 0.01	0.60 ± 0.04	0.59 ± 0.02	0.68 ± 0.04
Food conversion ratio (FCR)	3.35 ± 0.08	3.07 ± 0.16	3.37 ± 0.14	3.57 ± 0.12	3.36 ± 0.11	3.93 ± 0.17
Average daily weight gain (g)	0.043 ± 0.004	0.066 ± 0.004	0.073 ± 0.001	0.091 ± 0.00	0.086 ± 0.004	0.104 ± 0.004
Average daily length gain (cm)	0.038 ± 0.004	0.044 ± 0.004	0.057 ± 0.00	0.073 ± 0.00	0.061 ± 0.004	0.076 ± 0.00

The present study proved that the incorporation of *S. fusiformis* in the feed of *C. catla* considerably induced the growth in terms of specific growth rate, weight gain and length gain of the fingerlings.

Diet supplemented with *S. fusiformis* (5 g/kg) had significantly increases the growth and feed consumption rate as compared to fish fed with the control diet. The fish fed with spirulina (5%) had higher feed consumption (19.71g) than other experimental feeds. The present study showed that the dietary supplementation of *S. fusiformis* promoted fish growth. These results may be due to the improved feed consumption and feed digestibility. Moreover, *S. fusiformis* contains various nutrients especially vitamins and minerals that may support in fish growth promotion. These results correlates with other researchers (Amit *et al*., 2014) who have reported that feeding spirulina to fish improved survival and growth rates. The palatability of spirulina diet enhanced feed consumption which directly increased the growth. Scaria *et al*., (2000), have reported that, fish consumed more spirulina feed showed an increased growth when compared to other type of feed**.** Akter *et al*.,(2019) reported that supplimentation of arginine also increases growth performance of Nile tilapia (*Oreochromis niloticus*). In the present study, the possible role in the feed absorption of *C. catla* has been evaluated, and the results showed a substantial increase of absorption (18.00g) in the *S. fusiformis* (5%) fed fish group. Similar results were observed by Abdel-Daim *et al*.,(2020), that the spirulina showed effective performance in absorption of feed by carps and were due to its nutritional composition and bioactive compounds. Nakono *et al*., (2003) recorded that the lack of cellulose from the cellular surface of spirulina renders its easy digestion, thus increases the appetite of fish and improve feed intake and nutrient digestibility.

The maximum consumption rate (366.58mg) was noticed in fish consuming 5% spirulina diet. These results correlates with previous findings of Tongsiri *et al*.,(2010) who demonstrated the effect of dietary spirulina level on increased growth performance with increasing level of spirulina. The absorption rate was found to be maximum in 5% of spirulina feed (SF5), it was in accordance with Abdel-Tawwab and Ahamed (2009). The metabolic rate was high (263.20 mg) in 5% spirulina diet. The highest food absorption efficiency was found to be 92.11 % in SF5 feed. The highest gross conversion efficiency of food was found to be 19.52% in SF5 feed, which is significantly higher than the rest of the treatments. The results of present study is in accordance with Takeuchi *et al*.,(2002) who found that feed supplement with spirulina powder improved the food absorption efficiency and feed conversion efficiency.

Growth performance (LG, WG, FCR and SGR) increased with increasing *S. fusiformis* inclusion. This increase could possibly be due to the improved feed intake and nutrient digestibility. These results were in corroborates with those of Dawah *et al*.,(2002) who found that the addition of algae in fish diets improved growth performance in tilapia. Furthermore, the increasing level of spirulina in diet provided better growth rate compared to other commercial diets (Dernekbasi *et al*.,2010). The maximum growth of 6.22g was noted in SF5 (5%) fed fishes. This is evident that supplementation with *S. fusiformis* up to 5% did not show negative impacts on growth performance in *C. catla*. These findings are similar to that of Amer (2016), who proved that replacement of fishmeal with *S. fusiformis* up to 10% did not reduce growth rate and growth performance in *C. catla*. A tendency toward good growth performance at 5% recorded in the present study suggests that, unlike plant originated ingredients, the inclusion of *S. fusiformis* as a feed additive may enhance feed efficiency by increasing gut microbes colonization. Abdel Tawwab and Ahmed (2009) also recorded that the better growth and feed utilization of *O.niloticus* were obtained at 5.0gm of *Spirulina platensis* / kg diet.

A significant difference in length gain was found between treatment groups compared to control group, where the highest enhancement in length (4.53cm) was observed in fishes fed with SF5 (5%) diet. Present findings are in agreement with that of Kim (2013), who has reported that spirulina exhibited beneficial effects on length gain of fish fingerlings**.** The growth pattern of this study also proved that the length of fish has a close relationship with its weight gain. Similar type of findings were reported by Olufemi *et al.,* (2019) and it is evident that the availability of food and favorable growth environment determined the length and weight gain of *niloticus*.

The specific growth is the measuring tool of reflecting the fish health status under natural and experimental conditions. In the present study spirulina found to have potential effect on SGR, it was found to be 0.68 % higher in spirulina diet (SF5). Our results also correlated with the studies of Ramakrishnan *et al*.,(2008) who have found that the spirulina incorporated diet produced better SGR. The specific growth rate recorded in rohu improved with higher levels of Spirulina (25%) inclusion differs significantly from that of the control (Thakare *et al*., 2014). In the present study, it was observed that the length of *C. catla* was significantly increased with the addition of Spirulina in the feed. Increased specific growth rate was also observed by Saroch ., (2012) in fishes fed with 5% spirulina impregnated feed. Food conversion ratio (FCR) was also observed in fishes from 3.07 to 3.93 with the diet containing spirulina. The present studies are inconsistent with the reports of Nandeesha *et al*. (2001); Ghosh *et al*. (2003); Ramakrishnan *et al*., (2008) and Tongsiri., (2010). Feed supplemented with *S. fusiformis* powder promoted the feed conversion ratio (Amit *et al*., 2014).The FCR results in the present study are in accordance with the findings of Tan *et al*.,(2017) ,who has reported that the feed conversion ratio was significantly altered when fish meal was supplemented with *S. fusiformis* at 30% replacement level. FCR for other diets were ranged from 1.31 to 2.97.

CONCLUSION

From the present investigation, it is concluded that the optimized dietary level of dried *S. fusiformis* in the diet of *C. catla* (5gm/Kg) for 60 days positively enhances the growth performance, weight gain, enhanced length as well as its survival. The use *S. fusiformis* in the artificial diet can thus reduce the amount of incorporated fish meal which is presently as the main source of protein for the carp culture.

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