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Effect of Dietary Administration of Efinol® FG on Growth and Enzymatic Activities of *Channa striatus* (Bloch, 1793)

[1]R. Arthi Manju, [1]M.A. Haniffa, [1]S.V. Arun Singh, [1]C. Muthu Ramakrishnan, [1]M. Dhanaraj, [1]B. Xavier Innocent, [1]S. Seetharaman and [2]A. Jesu Arockiaraj
[1]Centre for Aquaculture Research and Extension, St. Xavier's (Autonomous) College, Palayamkottai, 627002 Tamil Nadu, India
[2]Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

Abstract: The probiotic Efinol® FG was added to basal diet (control) in four concentrations *viz.*, T1-10^3 cells g^{-1} incorporated diet; T2-10^4, T3-10^5 and T4-10^6 cells g^{-1} and supplied to the striped snakehead *Channa striatus*. *C. striatus* fed with probiotic diet showed significantly better growth performance ($p<0.05$) than those fed on basal diet alone. The total heterotrophic gut bacterial count in T4 was higher $7.7\pm1.89\times10^8$ CFU g^{-1} when compared to that of the other 3 trials as well as control diet. The mean digestive enzyme activity of all probiotic treatments was significantly different ($p<0.05$) from that of the control. The protease activity of 2, 3 and T4 was significantly >T1 and the control. Among the different probiotic trials no significant difference was noticed between 3 and T4. The amylase activity of T4 was the highest 68.71 ± 5.23 U mg^{-1} and significantly different ($p<0.05$) from that of the control 47.83 ± 3.75 U mg^{-1} and T1 49.97 ± 3.98 U mg^{-1}. All the probiotic treated *C. striatus* showed higher lipase activity than that of the control 78.93 ± 5.31 U g^{-1}.

Key words: *C. striatus*, Efinol® FG, growth, survival, digestive enzyme, heterotrophic

INTRODUCTION

Control of bacterial pathogens in fish farms has been routinely achieved by administration of antibiotics and chemotherapeutants. However, these were less successful due to the emergence of drug resistant strains (Thyssen and Ollevier, 2001). As a result, the modern aquaculture industry demands alternative prophylactics that may help to keep a healthy environment resulting in better production and higher profits. Within this context immunostimulants or probiotics seem to be very promising alternative (Balcazar *et al*., 2007). The research on probiotics for aquatic animals is increasing with demand for an eco friendly aquaculture (Verschuere *et al*., 2000).

Most of these studies have been related to challenge trials and suppression of pathogen by probiotics (Vaazquez *et al*., 2005) while another promising aspect is the use of probiotics in fish diets for potential improvement in feed efficiency.

Buts *et al*. (1999) have reported that in terrestrial animals the probiotics influence digestive processes by increasing the beneficial microbes thereby increasing microbial enzymatic activities and digestive enzyme activities. This results in higher digestibility of food and improved feed utilization (Bomba *et al*., 2002) and similar effect has been reported in some aquatic animals such as turbot larvae (Gatesoupe, 1991) and shrimps (Uma *et al*., 1999). Aquaculture diets are conventionally based on expensive feed stuffs such as fish and fish meal. Development of aquaculture will be greatly enhanced by finding alternative and less expensive ingredients. The culture of murrel *Channa striatus* is a very promising industry in Asian countries like India but the most serious constraints are non availability of seeds and lack of knowledge of feeding techniques.

Murrels are carnivorous, piscivorous and cannibalistic in nature. Feeding is a herculean task and hence fish farmers are facing problems due to non-availability of readymade feed. At this juncture the nutritional physiology of murrels is very much important to promote murrel culture among farms (Ghosh *et al*., 2002).

Efinol® FG, a commercial probiotic is a combination of highly concentrated thermostable beneficial microbes (*Bacillus subtilis*, *B. coagulans* and *Saccharomyces cerevisiae* along with selected nutrients, free flow and anti caking) that can withstand and high temperatures and function as growth promoters and immunomodulators. Efinol® FG is non-pathogenic, non-toxic and can survive in gut and remain stable and viable for long periods under storage and field conditions. Semi moist feeds are recently

Corresponding Author: C. Muthu Ramakrishnan, Centre for Aquaculture Research and Extension, St. Xavier's (Autonomous) College, Palayamkottai, 627002 Tamil Nadu, India

used in grow out culture of *C. striatus* because of their high feed conversion efficiency, easy preparation, digestion and better conversion ratio (Haniffa *et al*., 2002).

This study was intended to determine the effect of Efinol® FG on growth, feed efficiency, microbial community and enzymatic activities of the murrel *C. striatus*.

MATERIALS AND METHODS

C. striatus fingerlings (2.7±0.03 g) were collected from Centre for Aquaculture Research and Extension (CARE), Aquafarm. The fingerlings were randomly selected and distributed in cement tanks (1000) filled with water at a rate of 45 fingerlings per tank. Total 3 Replicates were maintained for each of the 5 treatments.

About 5 diets were prepared an unenriched control diet and four diets containing Efinol® FG. Efinol® FG was obtained from Bentoli AgriNutrition (USA). Lyophilized cells [10^{11} Colony Forming Units (CFU) g^{-1} of cells wet weight] were maintained at -20°C prior to use at a concentration of 10^3 CFU g^{-1} (T1), 10^4 CFU g^{-1} (T2), 10^5 CFU g^{-1} (T3) and 10^6 CFU g^{-1} (T4) (Table 1). The ingredients were mixed and the probiotics Efinol®FG was added to the autoclaved semi moist feed and kept at -20°C. The biochemical analysis of the feeds was analyzed by standard methods (AOAC, 1995). The fingerlings were fed 4% of their body weight thrice a day for 45 days. About 13rd of the water was changed daily. The temperature 26-28°C, salinity 27-28%, Total ammonia 0.02 mg L^{-1} and pH 7-7.3 were recorded. Fingerlings were weighed at 15 day intervals to determine weight gain, Specific Growth Rate (SGR), Food Conversion Ratio (FCR) and survival rate:

$$\text{Weight gain (g)} = \text{Final live weight (g)} - \text{Initial live weight (g)}$$

$$\text{Specific Growth Rate (SGR)} = \frac{\text{In log Final weight} - \text{In log Initial weight}}{\text{Time (days)}} \times 100$$

$$\text{Food Conversion Ratio (FCR)} = \frac{\text{Dry food consumed (g)}}{\text{Wet weight gain (g)}}$$

$$\text{Survival rate (\%)} = \frac{\text{Final number of fishes}}{\text{Initial number of fishes}} \times 100$$

Gut samples were well homogenized and serial dilution was performed under aseptic conditions, Total heterotrophic bacterial counts were recorded using Tryptic soy Agar plates. Selective media such as Sporulating agar (*B. subtilis*), Alkaline Bacillus media (*B. coagulans*) and Potato Dextrose Agar (yeast) were used and the observations were recorded as colony forming unit (CFU mL^{-1}). Protease activity was evaluated using Lowry *et al*. (1951) amylase activity following Jiang (1982) and Worthington and WBC (1993) and expressed as specific activity (U mg^{-1} protein)and lipase activity based on methods of Borlongan (1990) and Jin (1995) and expressed as U g^{-1}. Means and standard deviations were compared by one-way ANOVA. Duncan's multiple range test using SPSS (version 7) software was performed to find significant ($p<0.05$) differences in growth parameters.

Table 1: Proximate composition of selected ingredients

Ingredients	Control feed (%)
Fish meal, Anchovy	26.90
Soy flour	25.00
Jawala acetes sp.	20.00
Tapioca flour	10.90
Wheat flour	10.00
Sun flower oil	5.80
Mineral premix[a]	0.50
Monosodium phosphate	0.50
Aqua savor	0.30
Vitamin premix[b]	0.10
Ascorbic acid	0.01
Efinol® FG	10^3-10.00^6

[a]Mineral premix to supply the following elements (mg kg^{-1} diet): zinc (as sulphate) 72, iron (as sulphate), 36, manganese (as sulphate) 12, copper (as sulphate) 24, cobalt (as chloride) 0.6, iodine (as iodate) 1.2, chromum (trivalent as chloride) 0.8, selenium (as selenate) 0.2 and molybdenum (as molybdate) 0.2; [b]Vitamin premix: (mg kg^{-1}) vitamin B12, 0.1; nicotinic acid, 80.0: riboflavin, 50; pantothenic acid, 180; menadione, 40 folic acid, 6.0; biotin, 0.6; thiamin hydrochloride, 15; pyridoxine, 60; thiamin, 40; inositol, 400; astaxanthin, 60; choline chloride, 20.0; vitamin C, 250 and (IU) vitamin A, 6000; vitamin D3, 2000; vitamin E, 6000 IU

RESULTS AND DISCUSSION

The total heterotrophic count in the initial fish was 5.4×10^4 CFU g^{-1} and increased to 7.7×10^8 CFU g^{-1} in the T4 fish with 10^6 CFU g^{-1}. *B. subtilis*, *B. coagulans* and *S. cerevisiae* count was determined using their respective selective media. The total *B. subtilis* in Sporulating agar was 9.3×10^5 CFU g^{-1}. *B. coagulans* in alkaline bacillus medium was 9.5×10^5 CFU g^{-1} and *S. cerevisiae* in potato dextrose agar medium was 9.7×10^4 CFU g^{-1} (Table 2).

The total heterotrophic bacterial count differed significantly among Efinol® FG diets. When compared to control *C. striatus* fed diet with Efinol® FG in different concentrations showed better survival in Table 3. The fish readily accepted all 5 diets. The control fish had statistically lower growth and survival than fish fed with probiotics (Efinol® FG) enriched diets. No mortality was recorded in T4 group whereas in control *C. striatus* 12% mortality was shown in Table 3. After 45 days, there was a significant difference between the mean weights of treated *C. striatus* (T1-T4). The highest weight gain of 16.37±0.08 g was noticed in T4 (10^6 CFU g^{-1}). The weight

Table 2: Growth of total heterotrophic count and *B. subtilis*, *B. coagulans*, *S. cerevisiae* in selective media. All values are reported as CFU g^{-1} and ±indicates the standard deviation

Diets	Days	Tryptic soy agar	*B. subtilis*	*B. coagulans*	*S. cerevisiae*
Control	0	$5.4\pm1.52\times10^{4}$	$4.2\pm2.49\times10^{2}$	$5.4\pm0.99\times10^{2}$	No growth
	15	$6.2\pm1.02\times10^{3}$	$1.0\pm0.29\times10^{3}$	$5.1\pm0.91\times10^{2}$	$1.2\pm0.24\times10^{1}$
	30	$2.2\pm1.13\times10^{4}$	$6.2\pm0.97\times10^{3}$	$1.7\pm0.75\times10^{2}$	No growth
	45	$3.4\pm1.30\times10^{4}$	$4.8\pm0.94\times10^{4}$	$6.0\pm0.65\times10^{3}$	$3.4\pm1.05\times10^{2}$
T1	15	$5.5\pm2.31\times10^{4}$	$6.6\pm1.35\times10^{2}$	$8.1\pm1.21\times10^{2}$	$3.7\pm1.41\times10^{1}$
	30	$6.3\pm1.36\times10^{4}$	$4.7\pm1.52\times10^{3}$	$2.5\pm0.94\times10^{3}$	$2.5\pm0.92\times10^{2}$
	45	$1.4\pm1.11\times10^{4}$	$4.8\pm1.82\times10^{4}$	$5.7\pm1.32\times10^{4}$	$5.6\pm1.31\times10^{3}$
T2	15	$5.6\pm1.37\times10^{4}$	$2.9\pm2.77\times10^{3}$	$5.8\pm0.94\times10^{3}$	$1.2\pm0.97\times10^{2}$
	30	$4.6\pm1.08\times10^{5}$	$5.8\pm1.37\times10^{3}$	$6.1\pm1.56\times10^{4}$	$1.5\pm1.06\times10^{3}$
	45	$4.4\pm0.89\times10^{6}$	$5.2\pm1.27\times10^{4}$	$9.2\pm0.65\times10^{4}$	$5.8\pm1.25\times10^{3}$
T3	15	$5.7\pm1.75\times10^{6}$	$3.5\pm0.76\times10^{3}$	$4.3\pm1.56\times10^{3}$	$1.8\pm0.57\times10^{2}$
	30	$6.1\pm0.91\times10^{7}$	$4.2\pm0.23\times10^{4}$	$3.2\pm1.27\times10^{4}$	$2.4\pm1.58\times10^{3}$
	45	$6.8\pm0.32\times10^{8}$	$5.3\pm1.39\times10^{5}$	$8.5\pm0.75\times10^{5}$	$3.5\pm2.37\times10^{3}$
T4	15	$6.1\pm0.08\times10^{7}$	$4.8\pm1.03\times10^{4}$	$6.2\pm0.89\times10^{4}$	$1.9\pm0.58\times10^{3}$
	30	$3.6\pm2.80\times10^{8}$	$4.1\pm0.77\times10^{5}$	$1.4\pm0.49\times10^{5}$	$5.1\pm0.89\times10^{4}$
	45	$7.7\pm1.89\times10^{8}$	$9.3\pm0.80\times10^{5}$	$9.5\pm0.89\times10^{5}$	$9.7\pm0.68\times10^{4}$

Table 3: Growth performance of *C. striatus* fed with experimental diets

Growth parameters	Control	T1	T2	T3	T4
Initial weight (g)	2.76 ± 0.03^{a}	2.82 ± 0.02^{b}	2.85 ± 0.08^{b}	2.70 ± 0.08^{a}	2.88 ± 0.09^{a}
Final weight (g)	14.35 ± 0.36^{a}	15.20 ± 0.33^{b}	16.00 ± 0.15^{b}	16.53 ± 0.13^{b}	19.25 ± 0.12^{b}
W.G (g)	11.59 ± 0.17^{a}	12.38 ± 0.03^{b}	13.65 ± 0.14^{b}	13.83 ± 0.07^{b}	16.37 ± 0.08^{ab}
SGR	3.66 ± 0.16^{a}	3.75 ± 0.02^{b}	3.84 ± 0.02^{b}	4.03 ± 0.02^{b}	4.22 ± 0.09^{ab}
FCR	1.59 ± 0.09^{a}	1.44 ± 0.09^{b}	1.39 ± 0.03^{b}	1.33 ± 0.01^{b}	$1.28\pm0.09^{a,b}$
Survival (%)	88.00	96.00	96.00	98.00	100.00

The mean values having different superscripts in the same row are significantly different at $p<0.05$%, level and ±indicates the standard deviation

gain of test fish of all the treatments was significantly higher than that of the control (11.59±0.17 g). Values obtained for weight gain (12.4-16.4 g) and specific growth rate (3.8-4.2) of all probiotic treated groups were also significantly higher ($p<0.05$) than those of the control (11.59 and 3.66 g). Mean values of Weight Gain (WG) and SGR were significantly different ($p<0.05$) among the different treatment groups. The highest SGR was observed in T4 (4.22±0.09% day^{-1}) where the FCR was also the least (1.28±0.09%).

After 45 days of the experimental tenure the digestive enzyme activity of all trial groups showed significant difference when compared to that of the control. But there was no significant difference between T1 and T2 trials. The Protease activity was significantly higher (135.26±14.15 U mg^{-1}) in T4 followed by T3 (125.80±6.16 U mg^{-1}), T2 (119.35±6.13 U mg^{-1}) and T1 (109.44±5.23 U mg^{-1}). In the control the same was measured as 93.75±4.16 U mg^{-1}.

The average value of Amylase activity was significantly higher (68±5.23 U mg^{-1}) in T4 when compared to T1 (49.97±3.98 U mg^{-1}) and the control (47.83±3.75 U mg^{-1}). Similarly, the average value of lipase activity in the intestine of T4 *C. Striatus* was also higher (95.78±7.23 Ug^{-1}) (Fig. 1).

The probiotics (Efinol® FG) feed fed fishes exhibited superior growth performance in comparison to the control feed fed fishes. Similar, results have been reported in Seabass (*Dicentratrchus labrax*) by Carnevali *et al.* (2006) and in Indian major carps by Ghosh *et al.* (2003) and Swain *et al.* (1996) and in live bearing ornamental has fish (Ghosh *et al.*, 2007). Among the different probiotics diets T4 diet showed comparatively better growth performance than others. This finding corresponds well with earlier studies by El-Haroun *et al.* (2006) who found that commercial probiotics Biogen® in higher concentration resulted in better growth performance in *Oreochromis niloticus*. They observed higher SGR and optimum FCR in Biogen® incorporated feed. Ahilan *et al.* (2004) has also concluded that the application of probiotics showed higher SGR and survival in gold fish (*Carassius auratus*).

The composition of intestinal microbiota is highly variable depending on the developmental stage and the environmental conditions (Ringo and Birkbeck, 1999; Huber *et al.*, 2004). In the present study the final microbial count was highest in probiotics fed fishes in particular T4 diet fed fishes.

The results were supported by Ramakrishnan *et al.* (2008) and Wache *et al.* (2006) who report increased bacterial count in common carp (*Cyprinus carpio*) and rainbow trout fed with probiotics diets. Similarly, probiotics promoted colonization of bacteria in the fish gut for a prolonged period and had capacity to adhere and grow well *in vitro* in the intestinal mucus from turbot (Makiridis *et al.*, 2000). The probiont (*B. subtilis*, *B. coagulans* and *S. cerevisiae*) load was found higher in T4 diet. Similarly, Robertson *et al.* (2000) observed a

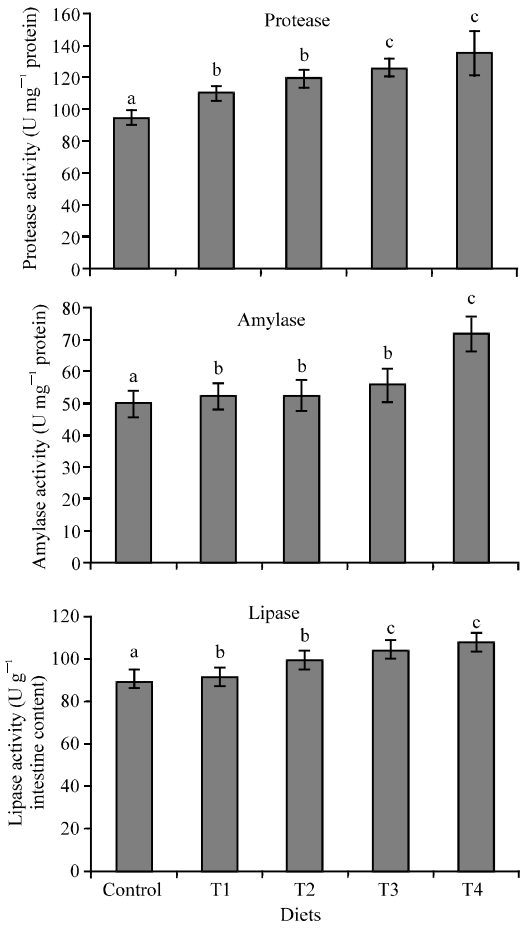

Fig. 1: Specific activity of protease, amylase and lipase in intestine content of *C. striatus* fed with control and four experimental diets containing probiotic Efinol®FG. Control, T1 Efinol® FG 103, T2 Efinol® FG 104, T3 Efinol®FG 105, T4 Efinol® FG 106 at the end of 45th day. Means with different superscripts were significantly different ($p<0.05$)

constant increase in Probiont (*Carnobacterium* sp.) population in the gut of rainbow trout and Atlantic salmon fingerlings fed with probiotic diet.

As supplementary components in aquaculture feeds, probiotics have strong adhesive and growth abilities (Mukhopadhyay and Paul, 1996). It can therefore, be inferred that Efinol® FG is effective in viably colonizing and proliferating in the digestive tract of host.

The effect of probiotics supplementation at intestine level was evaluated by the activity of enzymes viz, protease, amylase and lipase. In the present study, the enhanced nutrient and enzyme levels in by probiont addition led to increased food digestion and absorption which in turn led to better growth of the fishes ingesting the probiotics cells. A possible explanation proposed by procedure by several researchers is that probiotics actively procedure a range of relevant enzymes such as amylase, protease and lipase (Fuller, 1989; De Schrijver and Ollevier, 2000; El-Haroun *et al*., 2006) and stimulates the specific and total activities was found to be higher in T4 diet. Similarly, the increase in digestibility by enzyme activities through the use of probiotics be demonstrated in white Shrimp (*Litopenaeus vannamei*) by Lin *et al*. (2004), Rodriganez *et al*. (2009) in juvenile Senegalese sole (*Solea senegalensis*) and Ghosh *et al*. (2002) in rohu (*Labeo rohita*).

CONCLUSION

In the study, the findings showed that the commercial probiotics Efinol® FG (10^6 CFU mL^{-1}), when compounded with feed improved the growth performance and survival of murrel *C. striatus*. The increase in specific activities of enzymes coupled with the substitution of pathogenic microbes by beneficial probiont population in the intestine of probiotics feed fed fishes led to enhance of food which in turn contributed to the improved survival and growth of *C. striatus*.

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