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RESEARCH ARTICLE

INFLUENCE OF COMMERCIAL PROBIOTIC (BIFILAC) ON GROWTH, FEED UTILIZATION EFFICIENCY AND BIOCHEMICAL COMPOSITION OF MURREL, CHANNA STRIATUS

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Abstract

The present study was carried out to evaluate the influence of dietary supplementation of commercial probiotic (mixture of several strains of bacteria and yeast) on growth performance, feed utilization efficiency and biochemical composition of murrel, *Channa striatus*. The feeding trial was conducted for 60 days, *C. striatus* fingerlings with similar body weight (4 ± 1 gm) were distributed randomly into five treatment groups, which fed a feed containing probiotics in five different concentrations such as 0.1 %, 0.2 %, 0.3 %, 0.4 % and 0.5 %. The control group was fed without probiotics for the same period. The fishes were fed twice a day at a rate of 5% of their body weight. At the end of the feeding trial, maximum weight gain, length gain, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and feed efficiency (FE) were observed ($P < 0.05$) in the 0.5% probiotic diet fed fishes. FCR was highest in control group and PER and FE values were maximum ($P < 0.05$) in 0.5% Bifilac supplemented group, which was significantly different from other feeding groups. Biochemical composition such as protein, carbohydrate, fat, moisture and ash contents were estimated in the muscle tissues of *Channa fry* on the initial and final days of the experimental period. At the end of 60 days of feeding trial, the biochemical composition differs significantly ($P < 0.05$) among different experimental group fishes and maximum was observed in 0.5% of Bifilac diet fed group. These results clearly suggest that the inclusion of Bifilac at 0.5% level can improve growth performance, feed utilization efficiency and biochemical composition in *C. striatus* fingerlings and it can be used effectively as a probiotics for the use in aquaculture.

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Introduction:-

Air breathing fishes form about 13% of the marketable freshwater fishes in India and among them murrels belonging to the genus *Channa* are highly priced all over India. They are also prevalent in most southern and south-eastern Asian countries largely due to their good taste and fast growth as well as resistance to diseases, handling and tolerance to inferior water quality (Hossain et al., 2008). It is a popular farmed fish, preferred for its faster growth performance and delicate taste. This fish is of high nutritional value for human with a good essential amino acid and fatty acid profile. Unfortunately, there are no commercially available feed formulated for this species and only little

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empirical information on its nutritional requirements has been reported so far (Shan et al., 2016). Indian fish farmers are unable to culture murrels due to non availability of seeds as well as feed.

Probiotics are live microbial feed supplements that improve the health of host by modifying the gastrointestinal tract of the fish. Fish, being a hydrophilic animal rely solely on the environment, which filtering through the body and gill as fish performs its physiological function would benefit from use of probiotics. Probiotics enhance the nutrient utilization, modulate gut flora, inhibit the growth of pathogenic bacteria and improve growth and immune system of the fish (Ulukoy et al., 2017).

The common probiotics that are used for aquatic animals comprised of Lactobacillus, Lactococcus, Leuconostoc, Carnobacterium, Shewanella, Bacillus, Aeromonas, Vibrio, Enterobacter, Pseudomonas, Bifidobacteria, Clostridium and Saccharomyces (Nayak, 2010). Usually, probiotics are used by adding directly in the feed ingredients or by spraying in the prepared feed. According to the guidelines of Food and Agriculture Organization (FAO) and World Health Organization (WHO), probiotics should have the capability of surviving while passing through the gut as well as resisting the gastric juices and bile. Probiotics should have ability to flourish and settle in the gut, which should be safe and effective for the host species. The present study has been designed to investigate the Influence of commercial probiotic (Bifilac) on growth, feed utilization efficiency and biochemical composition of murrel, *C. striatus*.

Materials and Methods:-

Collection and Maintenance of Experimental fish:

Healthy *Channa striatus* fingerlings (average weight 4±1gm) were obtained from Bavani Sagar Dam at Erode, Tamilnadu, India. The collected fish was transferred alive in polyethylene bags and brought to the laboratory and acclimated for 2 weeks in rectangular cement tanks in non-chlorinated water and fed with basal diets twice daily before experimentation.

Preparation of experimental feeds:

The commercially available probiotic strain, Bifilac was selected and tested for their efficacy in the experimental fishes. Bifilac contains live cultures of *Lactobacillus acidophilus*, *L. rhamnosus*, *Bifidobacterium longum*, *B. bifidum*, *Streptococcus thermophilus* and *Saccharomyces boulardii*. Probiotic tablets were collected from the local pharmaceutical store, ground well using mortar and pestle and made into a fine powder. The feed ingredients used in fish feed were rice bran, groundnut oil cake, chicken waste, powdered probiotics and tapioca flour (as a binder). All the ingredients except probiotics were mixed thoroughly in a mix blender. The required amount of water was added to mixed ingredients to form soft dough. Then the dough was kept in an airtight polyethylene packet for one hour for proper conditioning followed by steam cooking for 20 minutes. Powdered probiotics were added to the feed after cooling, mixed well and passed through an extruder with 0.8 mm diameter holes. Pellets thus obtained were dried in the hot sun and stored in airtight polyethylene container to prevent fungal contamination.

Experimental design:

For the feeding trial, the fish were allotted to six tanks in a completely randomized design containing 20 fish per tank in triplicate. The fish were fed with different concentration of commercial probiotic, Bifilac included diets (0.1 %, 0.2 %, 0.3 %, 0.4 % and 0.5 %) twice per day at 5% body weight for 60 days. The control fishes fed with basal diet (not Supplemented with probiotics) were also maintained. Unfed and faecal matters were properly and water was changed on alternative days.

Growth parameters:

Weight and length of the control and experimental fishes were taken just before starting the experiment, followed by 15 days interval till the end of the experiment. The growth parameters in terms of weight gain, length gain and specific growth rate (weight) were evaluated as follows.

$$\text{Percentage weight gain} = \frac{\text{Final weight (gm)} - \text{Initial weight (gm)}}{\text{Initial weight (gm)}} \times 100$$

$$\text{Percentage length gain} = \frac{\text{Final length (cm)} - \text{Initial length (cm)}}{\text{Initial length (cm)}} \times 100$$

$$\text{Specific growth rate (weight) (\% day}^{-1}\text{)} = \frac{\text{In Final weight (gm)} - \text{In Initial weight (gm)}}{\text{Days of experiment}} \times 100$$

Biochemical analyses:

Biochemical compositions (protein, carbohydrate and fat) were analyzed in the control and probiotic supplemented feeds. Before starting the experiment and at the termination of 60 days of feeding trial, fishes from each experimental unit were analyzed for their biochemical composition such as protein (Lowry et al., 1957), carbohydrate (Hedge and Hofreiter, 1962), fat (Folch et al., 1957), moisture and ash (A.O.A.C., 1975) contents using standard procedures.

Estimation of feed utilization efficiencies:

Feed utilization efficiencies in terms of feed conversion ratio (FCR), protein efficiency ratio (PER) and feed efficiency (FE) were estimated after 60 days of the experimental period in *C. striatus* fed with control and different experimental feeds.

Feed conversion ratio (%)

$$\text{Feed conversion ratio} = \frac{\text{Feed given}^*}{\text{Weight gain}^{**}} \times 100$$

*As fed basis i.e., dry weight

** Wet or fresh weight gain

Protein efficiency ratio (%)

$$\text{Protein efficiency ratio} = \frac{\text{Weight gain (gm)}}{\text{Protein intake (gm)}} \times 100$$

Feed efficiency (%)

$$\text{Feed efficiency} = \frac{\text{Weight gain (gm)}}{\text{Feed intake (gm)}} \times 100$$

Statistical analysis:

Data obtained were analysed by one-way analysis of variance (ANOVA). The means among the treatments were compared using Duncan's Multiple Range Test using SPSS statistical package (Version 20.0, IBM SPSS Inc., NY, USA). Comparisons were made at the 5% probability level.

Results and Discussion:-

Probiotics supplementation (Bifilac) improved the growth performance, biochemical composition and nutritional indices of *C. Striatus* during the experimental period of 60 days.

Growth Performance:

C. striatus grown in different probiotic treatments (0.1% to 0.5%) exhibited highest growth rate than the control fishes. However, maximum weight gain (5.00, 8.20, 12.75 and 18.98%) and length gain (6.10, 8.24, 12.90 and 17.80%) were recorded in 0.5% Bifilac fed fishes during 15, 30, 45 and 60 days of the experiment. At the end of the experiment, minimum weight and length gain (16.42 and 14.60%) were recorded in the control fishes (Tables 1 to 3). Yanbo and Zirong (2006) working with common carp (*Cyprinus carpio*) fed diets supplemented with probiotics showed increase in the values of weight. The present results also supported by Ayoola et al. (2013), who reported that supplementation of *Lactobacillus* and *Bifidobacterium* increased the growth performance in *Clarias gariepinus* juveniles.

Bifilac (contain mixed cultures of bacteria and yeast) supplemented diet fed fishes (0.5%) showed highest specific growth rate (weight) during 15 days (3.55 %), 30 days (4.30%), 45 days (4.65%) and 60 days (%) of the experiment. Lowest specific growth rate (weight) values of 2.36 % were recorded in the control fishes at the end of the experimental period. Lara-Flores et al. (2003) reported that all the probiotic containing diets resulted in growth

higher than that of the control diets for tilapia (*O. niloticus* L.). They described that the addition of probiotics mitigated the effects of the stress factors and resulted in better fish performance, with better growth results in the diets containing the yeast. Similar results were observed when yeast was isolated from the intestines of wild rainbow trout. This probiotic showed a significant enhancement in growth of the cultured trout when it was introduced into the digestive tract of domestic rainbow trout (Gomez-Gil, 2000).

Kennedy et al. (1998) showed that the addition of a gram-positive probiotic bacterium increased the survival, size uniformity and the growth rate of marine fish larvae. Similar observations have been reported on *C. gariepinus* (Al-Dohail et al., 2009), *Labeo rohita* (Giri et al., 2013) and *Dicentrarchus labrax* (Carnevali et al., 2006). They reported that growth performance in the fishes were significantly ($P < 0.05$) better in the treated groups than the control when probiotics used as feed additives in their formulated diets.

Probiotics as feed supplements benefit the host by improving the feed value, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, antimutagenic and anticarcinogenic activity, growth promoting factors and increasing immune response. The improvement in growth may, however, be related to the improvement in the intestinal microbial flora balance as reported by Fuller (1989).

Biochemical composition:

Probiotics incorporation in the experimental feeds enhanced the biochemical composition in the muscle tissues of *C. striatus* after 60 days of experimental period. Highest moisture (75.84%), protein (23.30), carbohydrate (4.90%), fat (11.33%) and ash contents (3.10%) were estimated in 0.5% Bifilac fedfishes and minimum biochemical composition (70.12, 9.20, 2.10, 6.73 and 1.40%) were noticed in the control fishes respectively (Table 4). The administration of probiotics in the diet resulted in improvement in the protein and lipid content, *C. striatus*. Dayal et al. (2012) have reported the influence of different sources of dietary lipid on the growth, feed efficiency and survival of snakehead *C. striatus* grow-out. The better utilization of diet is reflected not only in specific growth rate but also in protein and energy efficiency values. Similar results were also supported by Munirasu et al. (2017), who observed that improved performance in growth and biochemical composition of freshwater fish *L. rohita* fingerlings.

Feed utilization efficiencies:

Feed utilization efficiencies were improved in the experimental fishes fed with different probiotics than the control fishes. Highest feed conversion ratio (0.57%) was recorded in the control fishes and 0.5% Bifilac fed fishes showed lowest feed conversion ratio (0.28%), which indicated better nutrient utilization (Table 5). Marzouk et al. (2008) studied the influence of some probiotics on the growth performance and intestinal microbial flora and observed higher feed conversion and productive performance in *O. niloticus*. The addition of probiotics could improve feed utilization even under stress conditions. The best FCR values were observed when probiotic-containing diets were fed to *C. striatus*. The use of *Spirulina* as a probiotic in Nile tilapia diet improved feed conversion ratio compared to the control (Abdel-Tawwab and Ahmad, 2009). In addition, Nile tilapia treated with commercial probiotic showed significantly higher feed conversion efficiency compared to the control (EL-Haroun, 2006). Furthermore, improved production was recorded for shrimp with significant lower feed conversion ratio than control using probiotic (Castex, 2008). Mohapatra et al. (2012) reported a lower FCR for rohu fingerling (*Labeo rohita*) when they fed the diets containing probiotics.

Maximum values of protein efficiency ratio (3.24%) and feed efficiency (59.20%) were recorded in *C. striatus* grown in 0.5% diet and minimum values of 1.20 and 20.10% were in the control fishes (Table 5). One of the most expected consequences of using probiotic bacteria is the direct effect of probiotic on the growth performance of fish either by direct increment in nutrient uptake, or by providing the nutrients. Several studies have demonstrated the positive effects of one most vital group of probiotic bacteria such as *Lactobacillus* species on the growth response of Nile tilapia freshwater prawn, gilthead sea bream, African catfish, Persian sturgeon and beluga fry. The reason of improved growth performance of cultured fish after feeding with probiotic diets might be due to improved quality and feed efficiency of diet, which ultimately stimulate the appetite of fish.

This results also agree with those obtained from previous studies by other authors (Lara-Flores et al., 2003), where growth, PER was reported to be better in Nile tilapia fed diets supplemented with probiotics than those fed a diet without probiotics supplementation. Similar observations have been reported on rainbow trout (Robertson et al., 2000), African cat fish (Al-Dohail et al., 2009). Some reports indicated that probiotics fortified diets could significantly improve the protein efficiency ratio or apparent nitrogen utilization (Kolndadacha, 2011).

Although different combinations of all the probiotics supplementation enhanced the overall growth performances of *C. striatus*, maximum growth, biochemical composition and feed utilization efficiencies were obtained in the 0.5% Bifilac supplemented fishes. Improvements in growth performance, feed and protein efficiency could be due to better nutrient digestibility, high-quality absorption and increased enzyme activities caused by a proper balance of the intestinal microbial flora (Fuller, 1989) Similarly, Suzer et al. (2008) and Wang et al. (2008) reported that digestive enzyme activities were increased when fish was fed with a probiotic-supplemented diet. Additionally, better growth performance and nutrient efficiency could possibly be related to lower stressor levels in fish fed the probiotics diet. It has been suggested that probiotics have a beneficial effect on the digestive processes of aquatic animals because probiotic strains synthesize extracellular enzymes such as proteases, amylases, and lipases as well as provide growth factors such as vitamins, fatty acids, and amino acids (Balcazar et al. 2008). Therefore, nutrients are absorbed more efficiently when the feed is supplemented with probiotics.

Table1:- Weight gain (%) in *C. striatus* during different days of the experiment in the control and probiotic supplemented feeds.

Feed	Concentrations (g kg ⁻¹)	Number of days during experiment			
		15	30	45	60
Control	---	3.66	6.70	10.78	16.42
Bifilac	0.1	3.72	6.98	11.00	16.97
	0.2	3.88	7.20	11.52	17.11
	0.3	4.25	7.87	11.94	17.96
	0.4	4.87	8.00	12.27	18.87
	0.5	5.00	8.20	12.75	18.98

Values are the mean of three replicates

Table2:- Length gain (%) in *C. striatus* during different days of the experiment in the control and probiotic supplemented feeds.

Feed	Concentrations (g kg ⁻¹)	Number of days during experiment			
		15	30	45	60
Control	---	4.12	5.12	9.80	14.60
Bifilac	0.1	4.58	5.84	10.60	14.92
	0.2	4.94	6.10	11.14	15.80
	0.3	5.12	6.94	11.80	16.66
	0.4	5.64	7.60	12.50	17.20
	0.5	6.10	8.24	12.90	17.80

Values are the mean of three replicates

Table 3:- Specific growth rate in percentage (weight) in *C. striatus* during different days of the experiment in the control and probiotic supplemented feeds.

Feed	Concentrations (g kg ⁻¹)	Number of days during experiment			
		15	30	45	60
Control	---	1.96	2.00	2.24	2.36
Bifilac	0.1	2.18	2.36	2.36	2.70
	0.2	2.46	2.90	3.40	3.10
	0.3	2.82	3.54	3.86	3.65
	0.4	3.28	3.92	4.20	4.10
	0.5	3.55	4.30	4.65	4.98

Values are the mean of three replicates.

Table 4:- Biochemical composition in muscle tissues of *C. striatus* before and after experimental period.

Feed	Concentrations	Moisture	Protein	Carbohydrate	Fat	Ash
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	(g kg ⁻¹)	(%)	(%)	(%)	(%)	(%)
Control	---	70.12	9.2	2.1	6.73	1.40
Bifilac	0.1	71.25	11.7	2.2	7.33	1.96
	0.2	72.25	13.9	2.7	8.46	2.26
	0.3	73.90	16.7	3.2	9.46	2.48
	0.4	75.00	18.6	3.7	10.43	2.80
	0.5	75.84	23.3	4.9	11.33	3.10

Values are the mean of three replicates.

Table 5:- Feed utilization efficiencies (%) of *C. striatus* in the control and probiotic supplemented feeds.

Feed	Concentrations (g kg ⁻¹)	FCR (%)	PER (%)	FE (%)
Control	---	0.57	1.20	20.10
Bifilac	0.1	0.54	1.64	28.68
	0.2	0.50	2.12	36.40
	0.3	0.42	2.48	44.60
	0.4	0.34	2.80	51.38
	0.5	0.28	3.24	59.20

Values are the mean of three replicates.

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