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RESEARCH ARTICLE

STUDIES ON THE EFFCET OF COMMERCIAL PROBIOTIC APPLICATION IN THE GROWTH OF THE FISH, *CYPRINUS CARPIO.*

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Abstract

Two types of Probiotic namely ***Lacto bacillus sporogenes*** and ***Bacillus subtilius*** (with concentration of 1500 million visible spores/gram of powder) were incorporated into mutagen formulated diets and fed to fish ***Cyprinus carpio*** (Weighed 6.0 to 7.0 grams) for 30 days. The commercial fish feed was incorporated to control sets without probiotic and mutagen incorporation. Among the two probionts tested, the high specific growth rate (SGR) at significant level 1% and 5% was observed in ***Lacto bacillus sporogenes*** then ***Bacillus subtillus*** fed fish respectively .This was due to low Food conversion Ratio (FCR),high protein intake and higher protein efficiency ratio (PER) in the ***Lacto bacillus*** incorporation compared to control fishes, and also fish showed the improved in the gut flora and microbial balance, this would have enhanced the digestibility and improved the metabolism in both probiotic applications compared to control, but there not much differences between the two treatments compared to control.

INTRODUCTION

Fishes are considered a delicacy and therefore have a huge demand in domestic and foreign markets. They are well known as a high protein, low fat food energy more or less 90-95 k.cal (Vasumathi , 2012). Aquaculture is a feed based industry with over 60 % of the operational cost come from feed source and the use of nutritionally balanced artificial feed with suitable management practices is a realistic approach to gain more genetic potential, better survival and higher growth in fishes (Ayyappan and Jena,1998).

Probiotics bacteria are now also being used in aquaculture by the way of following criteria: 1.porduction of inhibitory compounds 2. Competition for adhesion sites 3. Competition for nutrients .4.source of nutrients and enzymatic attribution to digestion 5.enhancement of immune response, 6.influence on water quality 7.interaction with phytoplankton and antiviral activity. Several probiotic species were used including *Lactobacillus* sp.(Jonnson 1986), ***Saccharomyces*** sp (Burnelt and Neil ,1977),***Bacillus*** sp,(Moriarty and Body , 1995; Morianty ,1998 ; Rengpipat *et al.*,1998 and Meunpal *et al.*, 2003). Vasumathi, 2012 , ***Lactobacillus*** It is apparent that the majority of probiotics for nutritional effect compares lactic acid bacteria notably ***Lactobacillus*** *spp*., ***Bifidobacterium*** *spp*., and *Streptococcus spp*., (Smoragieviez *et al* .,1993 and Gatesoupe ,1994), because lactic acid bacteria formed a major component of the normal micro flora in the gastro –intestinal tracts of healthy fish and mammals (Ringo and Gatesoupe ,1998).The effect of probiotics for fish and shrimp has been reported (Mohanty *et al* ., 2004, 2009; Wang *et al* .,2008;Wang and Xu ,2006). Further ,it is evident that most of the probiotics are used for shrimp and prawn culture (Indulkars and Belsare,2003; Venkat *et al* ;2004). In this concept the present study comparative aspects was carried out the impact of supplementation of diets with Probiotic *Lactobacillus* on the growth of fish ,***Cyprinus carpio*** in a laboratory condition .

Materials and Method

Fingerlings of ***Cyprinus carpio*** were bought from Tamil Nadu Aquaculture Farm , Mettur Dam –Mettur ,Tamil Nadu ,India in oxygenated polythene bag and transported to our college Laboratory,and acclimatized for 15 days in rearing cement tank (60 cm x 20 cm).During this period the fish were fed with ground nut oil cake and rice bran (1:1). Ninety Fishes were chosen from the stock and divided in to 3 groups .Each group is in turn divided in to 5 sets, and each set containing 10 fishes and introduced into plastic troughs in the capacity of 20 liters.

Probiotic feeding

The feed additives are *Lact-Act* and *Bioplus- 2B* (***Lactobacillus sporogenes*** and ***Bacillus stubtilis*** with a concentration of 500 million viable spores per gram of powder) and Thionil (mixture of bacterial culture)(Poseidon Biotech ,Chennai, Tamil Nadu ,India) and Mutagen includes Vitamin A,D,E,K,B_1,B_2,B_6,B_{12},Biotin ,Ascorbic acid, Iron ,Manganese , Copper ,Zinc ,Iodine ,Calcium ,Magnesium , Immuno stimulant and amino acid were mixed in the feed as per company feed direction broadcasted to the probiotic experimental groups.

Procedure Adopted for Probiotic Mixing

The known quantity of C.P. feed and the Lact act 10gm/kg , and Bioplus -25,Thionil 20g /kg and Mutagen 15g/kg were mixed with water and to this 30 ml of affinity gel like egg yolk also mixed and kept for 30 minutes ,dried in the shade for 20-30 minutes, then feed was broadcasted as per feeding schedules. Probiotic mixed feed was offered to fish 2 times /day.

Feeding Schedule

Commercial fish pelletized feed was given to the control groups without probiotic mixture. The size of pellet was ranged from 0.4-0.6mm. Biochemical composition of the pellet was given in table 1. Each set of animals were fed with 5%of initial body weight twice a day for 30 days. After the experimental period from each trough fish was taken, weighed, and then used for biochemical estimation (AOAC,1994).The growth measurement followed by Hussan and Jafri (1996).The unfed and faces were collected daily by manual siphoning ,dried and stored along with mixed probiotic thought the experimental period. The type of experimental diet of two probionts namely, ***Lactobacillus sporogenes*** and ***Bacillus sutibulis***, this was given in two different doses $68X10^7$ and $31X10^6$of each probiont .

Determination of Nutritional Effects and Survival

Every 15 days , fishes were taken to determine wet weight and total length .The numbers of mortality were recorded as well. Digital capillary to measure the length of the fish with 0.01 mm and digital scale to weight of fish with 0.01 g were used. Indicators of growth include: body weight increase (BWI), percent of body weight increase (PBWI%), specific growth rate (SGR),Feed conversion ratio (FGR)and Survival ratio (SR)were expressed as following

$BWI = W_t - W_0$

$$PBWI(\%) = 100^* \frac{W_{(t)} - W_{(i)}}{W_I}$$

$$SGR = 100^* \frac{InW_{(2)} - InW_{(1)}}{T2 - T1}$$

$$FCR = \frac{\text{Dry weight of ingested food}}{\text{Wet weight of produced treated}}$$

$SR = N^* W$

Where t is the period of culture in days, InW0 is the natural logarithm of the weight of the fry at the beginning of the experiment and In W_t is the natural logarithm of the weight of the fry at day t.(W_0 and W_t are in gram).

Statistical Analysis

The studied data were statistically analyzed for each series of the experiment, and the difference among the means were tested with Duncan's multiple range test (Snedecor and Cochran,1969).Data are presented as treatment mean $\pm$ SD .The values of $P < 0.05$ were considered significantly different .All analyses were performed using statistical software SPSS v.16.

Result and Discussion

Table:2.Over all growth performance of *Cyprinus carpio* fish fed with probiont and control group.

Feed Type	Initial weight (g)	Final weight (g)	weight gain	Specific growth ratio (SGR)	Food conversion ratio(FCR)	Protein efficiency Ratio (PER)	Protein Intake (PI%)
Control	325.72	345.62	19.9	0.98	1.61	1.29	13.26

(i) Probiont *Lactobacillus* $68X10^7$	428.15	575.48*	147.33*	1.79*	1.51*	1.86*	26.45*
$31X10^6$	432.13	581.23**	149.1**	1.89*	1.28*	1.58*	28.32*
Probiotic.ii *Bacillus subtilus* $68X10^7$	382.28	515.25*	132.99*	1.76*	1.78*	1.73*	16.52*
$31X10^6$	394.31	523.21**	128.9*	1.86*	1.52*	1.38*	15.12*

Statistically significant at $P < 0.05$, $P < 0.01$**

Table .3.Proximate analysis of muscle tissues of *Cyprinus carpio* fish fed with normal and Probiont feed.

Feed types	Carbohydrate(%)	Protein(%)	Lipid(%)
Initial fish	11.07	52.10	15.0
Control	13.72	53.00	16.12
Lactobacillus $68X10^7$	16.78 [a]	57.25[a]	17.84 [a]
$31X10^6$	18.09[a]	55.12[a]	16.91[a]
Bacillus subtilis $68X10^7$	15.10[a]	54.13 [a]	16.42[a]
$31X10^6$	15.23[a]	55.00[a]	16.40[a]

Mean values in the same column having different superscripts differ significantly ($P < 0.05$).

The growth performance result are given in the table 2 .The growth of ***Cyprinus carpio*** fish was highly influenced by probionts incorporated diets in all the experimental doses. The results indicated that more growth (SGR) and lowest (FCR) food conversion ratio was attained by the fish fed with ***Lactobacillus sporogenes*** followed by ***Bacillus stubtilis*** incorporated diet, when compared to control .In the present study, increased standard growth rate (SGR)and lesser FCR was found to be significant at 5% level against control and to the second group of treatments .From the above results, it could be presumed that the two dose concentrations of ***L. sporogenes*** and ***Bacillus stubtilis*** diets influenced the fish very much .Higher protein intake, protein utilization, and protein efficiency ratio (PER) also found in experimental diets which had higher SGR(Table 2).The results also proved that the probiont along with supplementary energy rich diets fed fish produced fatty fish with efficient protein utilization than control diets there by the probionts influence was found .obvious through mobilization of dietary fat. The present study was supported by Lu *et al*. (2002) and Sedhu Ramalingam *et al* . (2007) who have reported that the incorporation of *Spirulina* up to 50% level in the diet of silver sea bream South African abalone and larval tilapia found to increase protein intake, protein efficiency as well as protein utilization. Abraham *et al*.(1995) and Paulraj *et al* .(2008),observed a similar positive effect on addition of probiotic feed supplement *Lactobacillus* on the growth of shrimp and fresh water Prawn respectively with significant level of FCR than control groups.

Growth rate through out the experiment was improved in both probiotic treatment it can be certainly suggested that the more probiotic cells in diets and host intestine would necessarily result in the more improvement growth rate and survival . The present study results are in conformation with the study of Safoura Sedaghat *et al,*. (2012) who reported effect of Biopto- 2B on growth probiotic Zebra fish, ***Danio rerio.*** Further, the present study was related to Far *et al* ., (2009) who tested a commercial probiotic ***Bacillus stubtilis*** had a higher SGR and lowest FCR and higher growth performance by consistent application to the shrimp culture .In the present study, the resulted increased growth rate in probiotic culture may be due to the improved feed conversion via increased fatty acid oxidation and utilization of dietary energy (Moor *et al* ., 2000). Further ,conversion ratio may differ with a nutritional quality of the food ,although food conversion efficiency was reported to very with environmental conditions. In the present study ,the use of blunders like egg yolk, and cod liver oil provide with feed has influenced the adhesion of cells

into intestine and facilitate the colonization in gut and it was also due to that nutrient modification inside the gut through microbial balance and pool of amino acid liberated from the main source also evidenced (Mohamadllatha,2002 and Vasumathi, 2012).

In the present study, in-corpation of ***Lactobacillus sporogenes*** exhibited promising results even at low concentration which influenced the fat metabolism, improved the proteins utilization by increasing nutrient up take from the fat rich feed presumed that the probiont (living microbes)cell could modify the nature of the nutrients, and enhance the bioavailability by changing the dietary inclusion to be made suitable for conversion of protein in to body flesh . This results was corollary with a report of Tovar *et, al*.(2002) and Sedhuramalingam *et al*.(2007) in larval of sea boss and the fish, ***Cyprinus carpio*** respectively.

Lactobacillus sporogenes and ***Bacillus stubtilis*** were also known to provide a number of vitamins ,enzymes ,and even unidentifiably growth factor through intestinal colonization to improve microbial balance in the gut ,speeded up digestion, enzymes actively to enhance protein utilization and even they could modify nutrients appropriately needed not only to promote growth and also to strengthen immune mechanism of fish (Stanley *et al*., 1993,Sahoo *et al*.,1999,Abidi, 2003,Karunasagar 2001,Vasumathi 2012).

Among the two types of probionts tested (in two doses each)***Lactobacillus sporogenes*** incorporated diets fed fish produced fatty fish with more body protein. When dose increased in the diet fed produced linear increase in the body protein concentrations which ranked next to control diet fed fish .There was no significant differences in the body carbohydrate between the two groups of the test diet fed fishes when compared to control. Higher body protein and low body lipids in ***Cyprinus carpio*** fish (Table - 3) fed with ***Lactobacillus sporogenes*** and ***Bacillus stubtilus*** supplemented diets were in agreement with studies Watanabe *et al*.(1990),Liao *et al* .,(2002) in larval tilapia and Vasumathi (2012), in fresh water Prawn *m.rosenbergii*. From the above study ,it was evident that apart from protein and fat could be used in fish feed to promote protein utilization by incorporating specific concentration of probiotics more effectively.

Conclusion

Over all, the research of probiotics in aquaculture of India is still in its early stage and not much of commercial probiotic products were licensed in India so far. In future the application of probiont looks bright. The author found that supplementation diet with the proper density of commercial ***Lact Act*** and ***Bioplus- 2B*** probiotic could be beneficial for growth and survival of common carp, ***Cyprinus carpio***, especially in fast growing conditions ,where it would be essential to stimulate the precocious maturation of digestive system (Wache *et, al*. 2006).No clear negative effect of probiotic on ***Crprinus carpio*** was detected .

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