**STUDY ON FISH GROWTH BY USING DIFFERENT PROBIOTIC BACTERIA****Pitta, D.M. *Wadhai, V.S AND *Wadekar B.G.**

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ABSTRACT: The study was undertaken with aim to evaluate the effect of probiotic bacteria on fish growth and development. Food is essential to the growth and development of all living beings. As humans eat a wide array of foods and aquaculture fishes. High-quality animal protein is provided by aquaculture animals, which also improves consumer nutrition. Animal protein fish is a good source of vitamin B12 and omega 3 fatty acids. A variety of micronutrients, which lowers the risk of ischemic heart disease, and provides better health. Estimation of population and income is showing casing that future needs high amount of meat for human consumption. As probiotics are readily available in the market and aid in better growth rates, high survival rates, weight gain, an improvement in metabolic digestion, and expanded performance of fish growth. Moreover, it improves fish immune tolerance, their capacity to handle stress in aquaculture, and their resistance to pathogens.

Keywords: Probiotics, Fish, Lactobacillus sp., Water analysis, Fish feed,

INTRODUCTION

Food plays crucial role in growth and development of living things. As we human consume wide variety of food material and aquaculture animals (fishes). Since, fishes are the chief source of protein, they are the building blocks of our body. Everything is made up of proteins including enzymes, hormones, cell membrane, antibodies etc. Nowadays the demand for the fishes is at peaks and aquaculture is developing everywhere. However, aquaculture farming Is an expanding field for the protein production (Gongora 1998; Klaenhammer and Kullen 1999). In ornamental fish cultures, nursery ponds and in fish farming, probiotics can be an effective solution. The probiotics are either mixed in the water to provide a beneficial effect or fed as a farm of live or dry feed for the fish. There are several positive benefits of feeding probiotics, it helps to boost immunity and improvise the balance of microorganisms in the digestive system, improves digestive enzyme activities while also providing health-enhancing effects, improved immune system functioning, decrease pathogenic problems in the gastrointestinal tract (GIT), improved pressure acceptance and better survival rates. It offers growth-promoting nutrients such as amino acids, vitamins, fatty acids and general welfare. (S. Illanjiam et al. 2019).

Increasing the production of aquaculture food is challenging, because of increasing diseases, water pollution environmental disasters like, stress and temperature, unstable pathogenic resistances and microbial spoilage in aquaculture. All these factors majorly affect

the fish immune system and suffer from diseases which causes difficulties in aquaculture and fish. (EI- Haroun et al. 2006; Glencross et al. 2007). Hence, probiotics are primary approach towards the welfare of aquatic animals. Since, probiotics are easily available in market that helps in better growth rate, high survival rate, weight gain, increase in the metabolic digestive activity, enlarged performance of growth of the fish, it also boosts the immune tolerance of the fish, can able to manage the stress in the aquaculture and increases pathogenic resistance. For human consumption this well grown fishes are definitely a better diet. Fishes are full of nutrients, vitamins and protein enriched food, that is also a beneficial factor for the human welfare (Agrawal 2005).

Keeping the above in view the present dissertation work entitled “Comparative Study of Probiotics bacteria on fish growths” has been undertaken with the following aim and objectives:

1. Evaluating the effect of probiotic bacteria on fish growth and development
2. Checking for physical appearance in between prescribed time interval (every 3 days).
 - a) Weight
 - b) Height
 - c) Movement
 - d) Col

MATERIAL AND METHODS

MATERIALS

- 1) Three Fish Pots
- 2) Petri Plates
- 3) Weighing Machine
- 4) 10 or 30 cm Scale
- 5) Bore Water
- 6) pH strip
- 7) Thermometer
- 8) Soyabean Powder
- 9) Soya Chunks Powder
- 10) Darolac Sachet
- 11) Viabact Sachet

METHODS

Collection of Fish

The local Chandrapur “Umino aquarium and pet shop” store was visited to acquire three fish. “Molly” is the popular name for the fish *Poecilia sphenops*. For the experiment, fishes of three different colours were gathered i.e gold, black and platinum. Scientific names for the three fish are,

- 1) Golden salfine molly
- 2) Black molly
- 3) Platinum lyretail molly

Collection of fish feed

Fish food that was purchased at the “golbazar and bazar ward” of the local Chandrapur market.

- 4) Soyabeen 50gm
- 5) Soya chunks 20gm

6) Optimum fish food (Market feed)

1) Probiotic sachets purchased at the “Chandak medical Chandrapur”. The probiotic sachets contain live probiotic microorganisms. Darolac

2) Viabact

Darolac sachet- The *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Saccharomyces boulardii* are involved in the preparation of Capsule Darolac.

Viabact sachet- It contains *Streptococcus faecalis*, *Clostridium butyricum*, *Bacillus mesentericus*, and *Lactobacillus sporogenes* as major active ingredients. These act as probiotics which help in restoring the balance of good bacteria in the intestine.

According to Gatesoupe (1999), the first application of probiotics in aquaculture was in the mid-1980s (Kozasa 1986) and since then interest in such environment-friendly treatments has increased rapidly. The use of probiotics for disease prevention and improved nutrition in aquaculture has gained prominence in recent times due to an increasing demand for environment-friendly aquaculture (Vine et al. 2006). Common probiotic products used in aquaculture, such as *Bacillus* species, can improve water quality by reducing the number of microbial pathogens in ponds (Wang et al. 2008).

Preparation of fish feeding

Soyabean and soya chunks were ground into a fine powder and used to make fish feed in a 1:1 ratio. Darolac was weighed along with 1 g of the soyabean and soyachunks powder. The food was blended into a paste by adding 2 ml of distilled water to a 50 ml beaker containing feed. The paste food was passed through sieve and sun dried. The food was offered to the fish, food was fed 4% of body weight of fish, twice a day during the 15 day's experiment.

Analysis of water quality and monitoring

The home “bore” water had taken for this experiment, “Anchaleshwar ward near bagad kidki Chandrapur” under controlled temperature conditions. The water quality was analyzed, temperature tested of three pots. The experimental pots temperatures were identical to the control pot's temperature of 27-27.50C. When the pH (Potential of Hydrogen) of control and experimental tanks was measured, experimental tanks had a pH of 7.6 to 7.8 whereas control tanks had a pH of 7.7 to 7.8.

Fish Weight and Height Analysis

The study was carried out for 15-day's period, under controlled temperature conditions. At interval of every 3 days weight and height of the fish is measured. The fish were brought to the laboratory in a polythene bag with water, and the water-filled phytajar was placed on the weighing machine with the count set to zero. A fish was introduced to the phytajar, and its weight was noted. Using a scale measuring 30 cm, and removed the fish, waited 10 seconds (until the fish stopped moving), then put it on the scale to measure its height.

Fish Movement and Colour Analysis

Three experimental tanks were used to observe the movement of the fish. Darolac and Viabact diet-fed fish demonstrated better movement when compared to the control diet-fed fish. Color alterations were noticed; fish on the control diet didn't exhibit any variation, whereas fish in the experimental tank displayed a little variation in colour.

Data analysis by using statistical software

Each experiment was repeated twice and each determination was done induplicate. The data were examined by analysis of variance (ANOVA) using MINITAB21 at a level of significance of $p < 0.05$.

RESULT AND DISCUSSION

Collection Of Fish

Three fishes were collected from the local market of Chandrapur. (Poecilia Sphenops)

- 1) Golden salfine molly
- 2) Black molly
- 3) Platinum lyretail molly

Initial weight, height and colour were noted of the three fishes. Fish 1. Golden salfine molly

Initial weight	1.712 g
Initial height	4.3 cm
Initial colour	Golden

Fish 2. Black molly

Initial weight	1.726 g
Initial height	4.8 cm
Initial colour	Black

Fish 3. Platinum lyretail molly

Initial weight	1.915 g
Initial height	4.8 cm
Initial colour	Platinum

Preparation Of Fish Feed

"Optimum" meal is fed to the control fish. Likewise, the fish's food in the test tanks includes Darolac and Vibact. Darolac sachet contains 1.25 billion cells in 1gm including Lactobacillus Acidophilus, Lactobacillus Rhamnosus, Bifidobacterium Longum, Saccharomyces Boulardii.

Viabact Each capsule contains-

Streptococcus faecalis - 60 million Clostridium butyricum - 4 million

Bacillus mesentericus - 2 million

Lactic acid bacillus -100 million.

Prepared food in paste form, sieved and dried. 4% of the body weight of the fish feed to respective fishes (Photoplate 1)



Photoplate 1: Fish feed prepared by Microorganisms of Darolac and Viabact

Water Quality

Temperature and pH of the water has been recorded, The experimental pots temperatures were identical to the control pot's temperature at the morning and the evening time (Table 1).

Table 1: Temperature of Fish pots water

Sr. No.	Fish tank	Temperature ⁰ C (Morning)	Temperature ⁰ C (Evening)
1	Control	26 -26.5	27- 27.5
2	Darolac	26- 26.5	27- 27.5
3	Viabact	26- 26.5	27- 27.5

When the pH (Potential of Hydrogen) of control and experimental tanks was measured, experimental tanks had a pH of 7.6 to 7.8 whereas control tanks had a pH of 7.7 to 7.8 (Table 2)

Table 2: pH of Fish Pots water

Sr. No.	Fish tank	pH
1	Control	7.7 – 7.8

2	Darolac	7.6 – 7.8
3	Viabact	7.6 – 7.8

According to Griffin (1992), Plumb (1999) and Darwish et al. (2009), fish are susceptible at temperatures ranging from 15 to 30°C, and young fish are more severely affected than adults. Water quality was maintained at an acceptable range for the healthy growth of fish (Chapman 1992) during this study.

4.0 Comparison of Fish Feed by Increasing Weight

Weight was taken after every 3 days over a span of 15 days, observation and respective readings are as given in table 3, fig. 1 and Photoplate 2. The statistical method of one-way ANOVA is used to calculate the comparative analysis of weight. The comparative study has shown the significant p value < 0.05 and standard deviation was found to be 0.1631 by ANOVA (Table 4).

Table 3: Comparison of Fish Feed by Increasing Weight

Days	Market-fed	Darolac-fed	Viabact-fed
	F1 (g)	F2 (g)	F3 (g)
Day 1	1.712	1.726	1.915
Day 5	1.806	1.879	2.091
Day 8	1.832	1.902	2.155
Day 11	1.939	2.005	2.277
Day 15	2.04	2.144	2.446

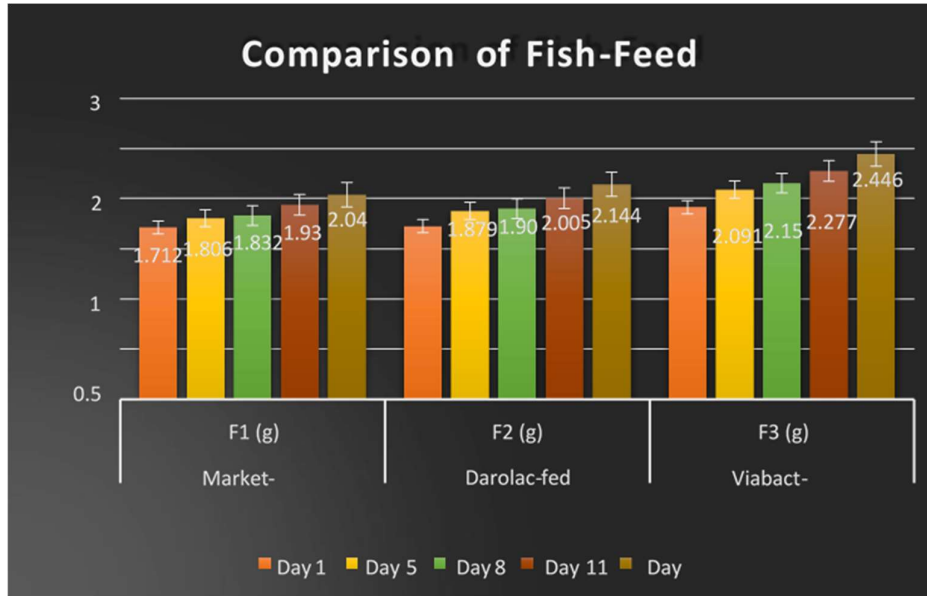


Fig. 1: Comparison of Fish Feed by Increasing Weight

Table 4: The statistical method of one-way ANOVA

One-way NOVA: F2(g), F3(g)

A F1(g),

	SS	MS	F	P	
Analysis of Variance					
Source	DF				
Factor	2	0.2689	0.1344	5.05	0.026
Error	12	0.3194	0.0266		
Total	14	0.5882			

Individual 95% CIs For Mean
Based on Pooled StDev

Level	N	Mean	StDev	-----+-----+-----+-----+-
F1(g)	5	1.8658	0.1266	(-----*-----)
F2(g)	5	1.9312	0.1553	(-----*-----)
F3(g)	5	2.1768	0.1993	(-----*-----)
				+ + + +
Pooled StDev =		0.1631	1.80	2.00 2.20 2.4

Honsheng (2010) worked on Tilapia fish by using the inclusion of *Bacillus subtilis*, who further attributed improved weight gain and feed efficiency to the increased enzyme production. Improved growth performance of Nile tilapia fed diets with *B. subtilis*, *Lactobacillus plantarum*, a mixture of *B. subtilis* and *L. plantarum*, and *Streptococcus cerevisiae* have been

reported by Essa et al. (2010). Aly et al. (2008a) compared the potential effect of two doses of *B. pumilus* and the commercial probiotic product Organic Green™ in improving immune response, survival, growth and resistance in Nile tilapia *A. hydrophila* infection after feeding for 4 or 8 weeks. Mean body weight and survival rates of all treatment groups showed statistically significant increases as compared to the control group. *S. faecium* + *L. acidophilus* or *S. cerevisiae* supplementation in tilapia diets containing 27% or 40% crude protein produced significantly higher weight gain and feed utilization efficiency compared to the control diet (Lara-Flores et al. 2010). Tilapia fed *S. cerevisiae* (Lara-Flores et al. 2010), *B. subtilis* + *S. cerevisiae* ((Lara-Flores et al. 2010; Marzouk et al. 2008), *Micrococcus luteus* (El Rhman et al. 2009), *Bacillus subtilis*, *Lactobacillus plantarum*, *B. subtilis* + *L. plantarum*, (Aly et al. 2008b; Essa et al. 2010), *Bacillus pumilus* (Aly et al. 2008b), *Lactobacillus acidophilus*, *Streptococcus faecium* (Lara-Flores et al. 2003), the commercial probiotic mixtures Organic Green™ (Aly et al. 2008c), Biogen® (El-Harounet al. 2006; Ghazala et al. 2010; Mehrim 2009), and Premalac® (El-Haroun et al. 2006) have all been shown to increase growth performance in tilapia.

As a result, the fish fed Viabact gained greater weight than those fed Darolac and control.



Photoplate 2: Comparative study on fish feed by Increasing weight

Comparison of Fish Feed on Fish by increasing Height

Height was taken after every 3 days over a span of 15 days, observation and respective readings are as given in table 5, fig. 2 and Photoplate 3. The statistical method of one-way ANOVA is used to calculate the comparative analysis of height. The comparative study has shown the significant p value < 0.05 and standard deviation was found to be 0.2366 by ANOVA (Table 6).

Table 5: Comparison of fish feed by increasing height

Days	Market-fed	Darolac-fed	Viabact-fed
	F1 (cm)	F2 (cm)	F3 (cm)
Day 1	4.3	4.8	4.8
Day 5	4.5	5	5.1
Day 8	4.7	5.1	5.3
Day 11	4.8	5.2	5.35
Day 15	4.9	5.3	5.5

Fig. 2: Comparison of fish feed by increasing height

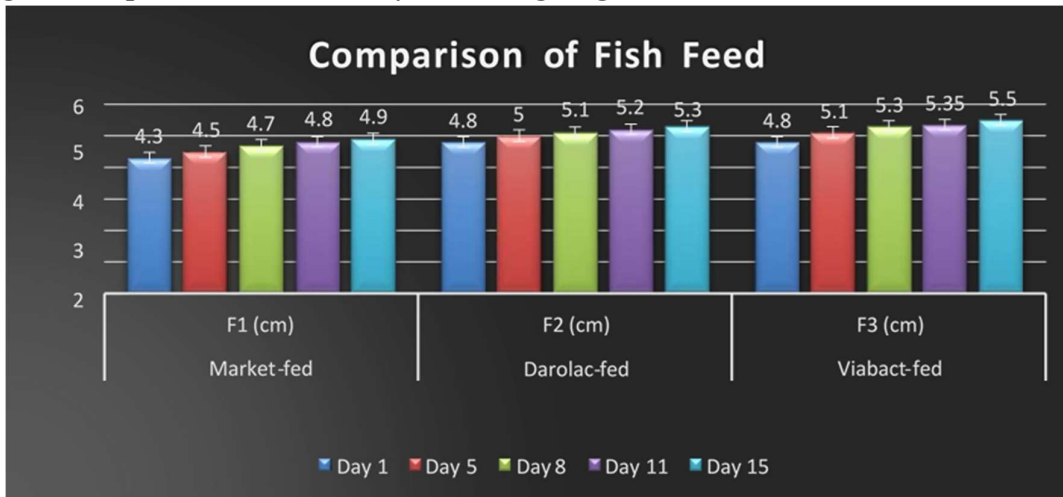


Table 6: The statistical method of one-way ANOVA

One-way NOVA: , F2 (cm), F3(cm)					
A F1(cm)					
Analysis of Variance	SS	MS	F	P	
Source	DF				
Factor	2	0.8923	0.4462	7.97	0.006

Error	12	0.6720	0.0560	
Total	14	1.5643		
				Individual 95% CIs For Mean Based on Pooled StDev
Level	N	Mean	StDev	-----+-----+-----+-----+--
F1(cm)	5	4.6400	0.2408	(-----*-----)
F2(cm)	5	5.0800	0.1924	(-----*-----)
F3(cm)	5	5.2100	0.2702	(-----*-----)
				----- + + + +
Pooled StDev =	0.2366	4.50	4.80	5.10 5.40

The use of probiotics for disease prevention and improved nutrition in aquaculture has gained prominence in recent times due to an increasing demand for environment-friendly aquaculture (Verschuere et al. 2000, Vine et al. 2006; Shelby et al. 2006; Wang et al. 2008; Cruz et al. 2012). A number of studies have reported the effectiveness of probiotics applied as water or feed additives in improving the immunity, health status, feed efficiency and growth performance of fish species with conflicting results (Boyd and Gross 1998; Verschuere et al. 2000; Aboagye 2008; Denev et al. 2009)

As a result the fish darolac and viabact gained the greater height than those fed with control.



Photoplate 3: Comparative study on Fish by Increasing height

Analysis of movement and colour

Movement and colour was observed of the three fishes in the 15 day’s experiment. Compared to fish provided the control diet, fish fed the darolac and viabact diet moved more freely. Fish on the control diet did not show any difference in colour, however fish in the experimental tank showed a slight variance in colour (Table 7).

Table 7: Movement and colour of Fish

Days	Control		Darolac		Viabact	
	Control (Movement)	Control (Colour)	Darolac (Movement)	Darolac (Colour)	Viabact (Movement)	Viabact (Colour)

Day-1	+	+	+	+	+	+
Day-5	+	-	-	-	-	-
Day-8	-	-	+	-	+	-
Day-11	-	-	+	+	++	++
Day-15	+	-	++	++	+++	+++

+ = Movement; - = No Movement; += Colour Change; -= No Colour Change;

CONCLUSION

The study was undertaken with aim to evaluate the effect of probiotic Bacteria on fish growth and development. Three fishes were collected and fed with probiotics in the result the fish fed with darolac and viabact showed better results in the comparison with control in height, weight, movement and even in colour variation.

As probiotics are readily available in the market and aid in better growth rates, high survival rates, weight gain, an improvement in metabolic digestion, and expanded performance of fish growth. Moreover, it improves fish immune tolerance, their capacity to handle stress in aquaculture, and their resistance to pathogens.

This study showed that, in comparison to market feed, fish fed probiotics had a substantial impact on their growth development. Hence, probiotics can serve as fishes main source of nutrition to produce a huge number of fish quickly. These well-grown fishes are unquestionably a superior diet for humans. Fishes are packed with vitamins, nutrients, and protein-rich food, which is also good for human welfare.

Final Weight of Market- fed fish was found to be 2.040 grams. Final Weight of ViBact- fed fish was found to be 2.446 grams. Final Weight of Darolac- fed fish was found to be 2.144 grams.

From above observations and finding we can conclude that highest weight gain observed in Viabact fed MOLLY fish whose weight was found to be 2.446 grams.

Microorganism found or used in Viabact is *Streptococcus faecalis*, *Clostridium butyricum*, *Bacillus mesentericus* Lactic acid bacillus.

Precaution to be taken while keeping a fish:

- Properly condition your water.
- Acclimate your fish to the water.
- Float fish in their bag.

- Maintain pH balance and other chemical levels.
- Make sure water temperature is right.
- Change water regularly.
- Clean tank glass and other structures.
- Choose the right size for your tank.
- Avoid overfeeding your fish

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