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Effects of dietary administration of probiotics (*Bacillus subtilis*) on the growth parameters of the fish *Labeo rohita*

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Abstract
In this study, we have evaluated the effects of dietary probiotic bacteria (*Bacillus subtilis*), on the growth parameters of the fish *Labeo rohita*. The probiotic (*Bacillus subtilis*) was procured from the market and mixed with the basal food in different combinations according to experimental need. In the present study, feeding trials were conducted in for 90 days in triplicate. For this feeding trial, live *Labeo rohita* were captured from the local fish farm located in Bareilly district. Fishes of similar body weight were distributed randomly into five glass tank that were continuously aeriated at Department of Zoology, Bareilly College, Bareilly, U.P. These five fish treatment groups were given five concentrations of *Bacillus* subtilis *viz*, 0.0 (T_1), 0.5(T_2). 1.0 (T_3) 1.5 (T_4) and 2.0 (T_5) with basal feed. T_1(0.0) was the control group that was fed on basal food only, without *Bacillus subtilis* for the same period. The effects of the probiotic (*Bacillus subtilis*) were analyzed by observing body length and weight after an interval of 15 days (at 0,15, 30,45, 60,75 and 90 days). Two treatment groups (T_4 and T_5) showed better growth of fishes, than any other treatment group. These results suggest that *Bacillus subtilis* can be an effective fish feed probiotic in the concentration 1.5% or 2% mixed in the basal feed for *Labeo rohita* in aquaculture practices.

Keywords: Probiotic, *Bacillus subtilis*, Growth Performance, *Labeo rohita*

1. Introduction
Nearly 17% of the animal protein intake of human being is provided by the aquaculture (Shah and Mraz 2020) [23]. Different types of nutrients particularly essential amino acids and fatty acids that our body needs are provided by the fishes. Vitamins and essential minerals like iodine and selenium that are very rarely present in other food sources are also provided by fishes. (Kwasek *et al*., 2020) [15]. Higher quantity of fat-soluble vitamins A, D, E, and K, is present in fish liver oil as well as fishes are good source of vitamin B complex, (Mohanty BP., 2015) [17]. Rohu, *Labeo rohita* is a very important fish species in India (FAO, 2001). It is an important species of carp polyculture system with catla (*Catla catla*) and mrigal (*Cirrhinus mrigala*). *Labeo rohita* is a graceful Indo-Gangetic riverine species. It is an important commercially cultured fish in India that has vast market demand due to its good taste and healthy flesh. In south India, it provides a major portion of the freshwater fish production. In aquaculture, fish diseases are considered as a serious problem and these are spread worldwide (Boonthai *et al*., 2011) [4]. In India infection of bacteria is the most important cause of diseases in aquaculture, particularly in the culture of catfish (Kesarcodi-Watson *et al*., 2008) [13]. In aquaculture the use of antibiotics for the treatment of bacterial infection and to prevent fish mortality is reducing day by day as pathogens become able to develop resistance against antibiotics (Nikoskelainen *et al*., 2003) [20]. So that the use of probiotics in aquaculture is expected as an excellent strategy to prevent the infectious diseases as probiotics have potential to replace antibiotics and other harmful chemicals (Balcazar *et al*., 2006) [2]. "Probiotics are live microbial feed supplement that beneficially affects the host animal by improving its intestinal balance". Today, probiotics are used as functional foods that improve the health and also used as growth supplements for human health as well as for animal production and growth (Mombelli, 2000) [18]. "*Bacillus subtilis* is a spore producing multifunctional probiotic bacterium.

It is recommended to support environmental, nutritional and metabolic changing conditions. *Bacillus subtilis* has versatility of growth nutrients utilization, high level of enzymes production, secretion of antimicrobial compounds, develops in aerobic and anaerobic conditions. *Bacillus subtilis* is generally recognized as safe (GRAS) by the Food and Drug Administration (FDA).

Materials and Methods Experimental design

For the present experiment *Labeo rohita* of similar size and weight were collected from the local fish farm in the Bareilly district. These fishes were distributed in the five experimental groups (*viz* T_1, T_2, T_3, T_4, T_5) and were stocked in properly washed five glass aquaria at Department of Zoology, Bareilly College Bareilly, UP. These experimental fish groups were acclimatized for 15 days before experimentation. Aerators were connected to fish aquaria for continuous aeriation and to maintain dissolved oxygen level in the water. Fishes were provided with basal diet during the acclimatization twice daily. There were 10 fishes per aquarium and each aquarium contain 70 liter water. Thus, the fishes with similar weights were collected to stock in 6 different aquaria (n 10 fishes/aquarium containing 70 liters water). Water quality parameters *viz* temperature, pH, and dissolved Oxygen were observed at regular interval with the help of digital thermometer, universal pH meter and Winkler method. Temperature was maintained between 18 - 28 ^{0}C, dissolved oxygen between 5.5 - 6.5 ppm and pH between 7.20 - 8.0.

At the starting of experimental period, length and weight measurement of all the experimental fish groups was done and recorded. In the control group (T_1) fishes were fed with basal feed only with 0% probiotics (*Bacillus* subtilis) for 90 days. While in other four groups, the fishes were fed with basal feed that have concentration of probiotic (*Bacillus* subtilis) 0.5% (T_2), 1.0% (T_3),1.5 (T_4) 2.0% (T_5), respectively, for 90 days.

Table 1: Percentage composition of experimental diet

S. No.	Treatment	Percentage of different ingredients					
		Rice Bran	Soya bean meal	Mustard oil cake	Wheat flour	Vitamins and minerals	*Bacillus* subtilis
1	T_1(Control)	38.0	25.0	25.0	10.0	2.0	0.0
2	T_2	37.5	25.0	25.0	10.0	2.0	0.5
3	T_3	37.5	24.5	25.0	10.0	2.0	1.0
4	T_4	37.5	24.5	24.5	10.0	2.0	1.5
5	T_5	37.5	24.5	24.5	9.5	2.0	2.0

The length and weight of each experiment group were recorded at 0, 15, 30, 45, 60, 75 and 90 days of experimental period by using electronic balance and standard scale. Parameters of growth performance were determined using standard formulae as following.

1. Net Length gain = Final length - Initial length

2. Percentage Length Gain = 100 x (Final Length - Initial length) / Initial Length

3. Net Weight gain = Final weight - Initial weight

4. Percentage Weight Gain = 100 x (Final weight - Initial weight)/ Initial weight

5. Survival rate = 100 x number of fishes recovered / Number of fishes stocked

6. Feed Conversion Ratio (FCR) = Total dry feed intake (gm) / wet weight gain (gm)

7. Specific growth rate (SGR) = 100 x In (Final body weight) - In (initial Body weight)/ Number of days.

Results were calculated using standard statistical procedure.

Observation and Results: In the present study, effect of different combinations of probiotic diet (0%, 0.5%, 1, 0%, 1.5%, 2.0%) was observed on the growth of the fish *Labeo rohita*. The composition of the basal diet and concentration of probiotic (*Bacillus subtilis*) in five experimental feed is given in table 1.

Net weight gain(gm), % weight gain, Net length gain (cm), % length gain in *Labeo rohita* fed with different concentration of probiotic (*Bacillus subtilis*) are given in table 2. Specific growth rate (SGR), Feed conversion ratio (FCR) and survival rate with different experimental diet are given in table 3.

From the data given in the table 2, We can conclude that highest weight gain was found in T_4 treatment group (64.40gm) followed by T_5(59.91). Percentage weight gain was highest in Treatment T_4 (405.54%) followed by T_5 (375.14%). Net length gain was highest in treatment group T_4 (9.35 cm) followed by T_5 (8.58 cm). Maximum percentage length gain was found in group T_4 (86.33%) followed by T_5 (78.93%). From these observations we can conclude that 1.5% concentration of probiotic *Bacillus subtilis* (T_4) is more favorable for maximum growth in *Labeo rohita* in control laboratory condition.

Table 2: Growth Performance of Rohu (*Labeo rohita*), fed with different experimental diet (mean ± SD)

Treat-ment	Fish weight (g)		Net weight gain(g)	% weight gain	Fish length (cm)		Net length gain (cm)	% length gain
	initial	Final			initial	final		
T_1	15.69 ± 0.36	60.64 ± 1.31	44.95	286.48	10.60 ±0.29	16.76 ±0.47	6.16	58.11
T_2	15.85 ±0.43	69.84 ±1.03	53.99	340.63	10.77 ±0.34	18.52±0.43	7.75	71.95
T_3	16.32 ±0. 34	74.95± 1.08	58.63	359.25	10.91 ±0.39	19.25 ±0.44	8.34	76.44
T_4	15.88 ± 0.53	80.28±1.28	64.40	405.54	10.83 ±0.34	20.18 ±0.49	9.35	86.33
T_5	15.97 ±0. 61	75.88 ±1.26	59.91	375.14	10.87 ±0.36	19.45 ±0.42	8.58	78.93

Table 3: Growth Parameters of *Labeo rohita* fed with different experimental diet

Treatment	Specific Growth Rate	Feed Conversion Ratio	Survival rate
T_1	1.50	3.05	83.33
T_2	1.64	2.85	86.66
T_3	1.69	2.80	93.33
T_4	1.80	2.68	100
T_5	1.73	2.75	100

Data regarding growth parameters is given in table 3. In these observations highest specific growth rate was recorded in T_4 (1.80) followed by T_5 (1.73). The highest feed conversion ratio was observed in T_1 (3.05) Followed by T_2 (2.85). Survival rate was maximum in T_5 (100) and T_4 (100).

Discussions

The use of probiotics in aquaculture shows promise, but continuous and quality research is needed. However, a number of products have been evidenced their efficacy and possible use in aquaculture. Probiotics help to boost appetite in the fishes due to detoxification of feeds and decomposition of undigested compounds (Irianto A and Austin B., 2002) [12]. Microorganisms have high rate of replication in the gastro-intestinal tract (GIT) so that they can colonize in it after prolonged administration. Probiotics help to maintain fish health by improving a few immunological characteristics and reducing pathogen burden on intestinal mucus layer as they cover the visible surface (Banerjee *et al*., 2017) [3]. Microorganisms are present in large numbers within the intestine of fishes and are generally considered harmless (Giri *et al*., 2013) [10].

The rise in nutrition digestibility is caused by the high number of digestive enzymes (protease, amylase, cellulose, phytase, etc.). These enzymes are released by the probiotic microorganisms associated within the intestines of the host (Ghosh *et al*., 2017) [9]. Bacterial probiotics prevent the colonization of pathogens by the physical competition for adhesions in the gut mucosal layer of the host. It is necessary to adhere in the gut mucosa of host for bacteria to remain in the intestines of aquatic animals (Cruz *et al*., 2012; Roesler *et al*., 2011) [6, 22]. Probiotics reduces the effects of disease and bacterial infection as they perform a beneficial function as immunostimulatory and assist protection in aquatic species (Dawood and Koshio, 2016) [7]. Therefore, probiotics work as immunostimulants and boost the immune response, resistance to disease. Due to this it help to sustain in the aquaculture. (Wu *et al*., 2015) [24]. The probiotic type *Bacillus subtilis* E20 improves antibacterial activities in grouper fish (Liu, *et al*. 2012) [16]. According to the recent studies higher rate of hatching and fast embryonic development is achieved in Zebrafish by the administration of probiotics (Gioacchini *et al*., 2013) [11].

Besides of these beneficial effects, Probiotics also have some limitations as follows: A few probiotic strains may also elevate the histamine levels in the fishes: it is a molecule which is produced by the immune system when a threat is being sensed. Due to increased concentration of histamine, the blood flow also increases, that is results into the redness and swelling in the infected tissue (Branco *et al*., 2018) [5]. Increase in the number of probiotic bacteria more than a limit in the body may cause toxicity and results into the organ failure or death also (Kothari *et al*., 2019) [14]. In the present study, probiotics show positive effect on the body weight of the fish *Labeo rohita* in the treatment T_4 and T_5 in comparison the control (T_1). It may be due to increase in the activity of digestive enzyme in the gut.

Conclusion

In the present study it is observed that feed with different concentration of probiotics (*Bacillus subtilis*) help to improve growth in the fish *Labeo rohita*. The experimental diet with 1.5% and 2.0% *Bacillus subtilis* is more suitable for fish growth. It is because of increased enzymatic activity and disease resistance in the fishes. So that probiotic can help to increase fish growth but continuous and quality research is needed.

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