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Effects of dietary administration of probiotic (*Bacillus subtilis*) and iron oxide nanoparticles on the growth parameters of the fish *Labeo rohita*

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Abstract
The effects of feed rich in iron oxide nanoparticles and the probiotic *Bacillus subtilis* on the growth and development of the fish *Labeo rohita* were investigated in this study. Depending on the needs of the experiment, different combinations of iron oxide nanoparticles and the probiotic *Bacillus subtilis* were added to the baseline feed. For ninety days, feeding studies were carried out in triplicate. Fish of approximately similar body weights were randomly distributed in five glass tanks that were continually aired at the Department of Zoology, Bareilly College, Bareilly, Uttar Pradesh, for this experiment. Five concentrations of iron oxide nanoparticles (i.e., 0 mg/kg, 15 mg/kg, 30 mg/kg, 45 mg/kg, and 60 mg/kg) and probiotic *Bacillus subtilis* (05%, 0.5%, 1%, 1.5%, and 2%) were administered concurrently to these five fish treatment groups. Treatment group T1, which served as the control group, was given a basal meal consisting of 0.0 mg/kg of iron oxide nanoparticles and 0% probiotic *Bacillus subtilis*. After a 15-day interval, body length and weight were measured to assess the effects of the probiotic *Bacillus subtilis* and iron oxide nanoparticles (*viz* 0, 15, 30, 45, 60, 75 and 90 days). Compared to other treatment groups, two treatment groups (T_4 and T_3) shown superior fish development. In order to improve the effectiveness of fish feed, 1.5% probiotic *Bacillus subtilis* and 45 mg/kg Iron oxide nanoparticles together in the basal diet of *Labeo rohita* is effective in aquaculture practices.

Keywords: Growth performance, iron oxide nanoparticles, *Bacillus subtilis*, probiotics, and *Labeo rohita*

Introduction

A variety of protein sources are found in for humans. Because their health benefits, aquatic proteins are preferred. In every nation, fish are an integral component of the human diet (Mohanty, 2015) [17]. Approximately 17% of the animal protein consumed by humans comes from aquaculture (Shah and Mraz 2020) [21]. So that the aquaculture industry contributes to livelihoods, food security, and nutrition. Fish also supply essential amino acids, vitamins, and important minerals like selenium and iodine (Kwasek and others, 2020) [16]. One significant fish species used in polycarp culture in India is Rohu, Labeo rohita (Hamilton, 1822) (FAO, 2001). It is a significant fish in the freshwater. Fish diseases are a major concern in the global aquaculture (Boonthai *et al*., 2011) [4]. One of the main causes of illnesses in fish culture is bacterial infection (Kesarcodi-Watson *et al*., 2008) [14]. Antibiotics are used in aquaculture to treat bacterial infections and to lower fish mortality rates, however bacteria can become resistant to antibiotics (Nikoskelainen *et al*., 2003) [19]. In order to prevent bacterial infections, probiotics are employed (Balcazar *et al*., 2006) [3].

"The Greek words pro and bios mean simply life, and this is where the term probiotic originates." (1999, Gismondo *et al*.) [9]. According to Fuller (1989) [7], the most widely used definition is "Probiotics are live microbial feed supplements that improve the intestinal balance of the host animal." *Bacillus subtilis* is a multipurpose probiotic that produces spores.

U.S. National Nanotechnology Initiative (NNI) define nanotechnology as "Understanding and control of matter at dimensions of roughly 1 to 100 nm where unique phenomena enable novel applications". "The study, design, creation, synthesis, manipulation and application of functional materials, devices, and systems through control of matter at the nano-meter scale (1-

100 nano-meters, one nano-meter being equal to 1 x $10^{-9}$of a meter), that is, at the atomic and molecular levels, and the exploitation of novel phenomena and properties of matter at that scale," is another way to put it simply.

Certain physiological activities, such as cellular respiration, oxygen transport, and lipid oxidation reactions, depend on iron (Andersen *et al*., 1997) [2]. According to Huber (2005) [13], iron oxide nanoparticles have a lot of promise for use as food additives, antibacterial additives, and in human medicinal applications. When compared to other forms of iron, dietary delivery of iron oxide nanoparticles is the most bioavailable form (Stephen, 2007) [22].

Method and Materials

Fish *Labeo rohita* of approximately similar size and weight were used in the five experimental groups (T1PN, T_{10}, T_{11}, T_{12}, and T_{13}) for the current study. These fishes were cultured in five adequately cleaned glass aquariums at the Department of Zoology, Bareilly College, Bareilly, Uttar Pradesh. Prior to experimentation, the fish underwent a 15-day acclimation period. Fish aquariums have aerators attached to them in order to maintain the water's dissolved oxygen level. During the acclimation stage, the fishes were fed a basal feed twice a day. There are 10 fish and 70 liters of water in each aquarium. As a result, fish were added to five distinct aquariums (10 fish per aquarium, holding 70 liters of water). At regular intervals, the temperature, pH, and dissolved oxygen were measured. A digital thermometer, and a universal pH meter, Winkler method were used to measure the temperature, pH, and dissolved oxygen. The temperature ranged from 18 to 28 ^{0}C, the dissolved oxygen ranged from 5.5 to 6.5 ppm, and the pH ranged from 7.20 to 8.0. At the beginning of the experiment, the length and weight of every fish were measured. Over the course of 90 days, the five experimental groups were fed varying concentrations of iron oxide nanoparticles (0 mg/kg (T1PN), 15 mg/kg (T_{10}), 30 mg/kg (T_{11}), 45 mg/kg (T_{12}), 60 mg/kg (T_{13})) and probiotics, *Bacillus subtilis*, at concentrations of 0% (T1PN), 0.5% (T_{10}), 1.0% (T_{11}), 1.5 (T_{12}), and 2.0% (T_{13}) mixed with basal diet together.

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	Iron oxide Nanoparticles (mg/kg)
1	T1PN (Control)	38.0	25.0	25.0	10.0	2.0	0.0	00
2	T10	37.5	25.0	25.0	10.0	2.0	0.5	15
3	T11	37.5	24.5	25.0	10.0	2.0	1.0	30
4	T12	37.5	24.5	24.5	10.0	2.0	1.5	45
5	T13	37.5	24.5	24.5	9.5	2.0	2.0	60

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.

Observations and Results: The growth of the fish *Labeo rohita* was examined in the current study to determine the effects of various combinations of probiotic *Bacillus subtilis* diet (0, 0.5, 1, 0, 1.5, 2.0%) and iron oxide nanoparticles (0 mg/kg, 15 mg/kg, 30 mg/kg, 45 mg/kg, 60 mg/kg) simultaneously. Table 2 shows the net weight increase (gm), percentage weight gain, net length gain (cm), and percentage length gain in *Labeo rohita* fed with varying concentrations of Iron oxide nanoparticles and probiotic (*Bacillus subtilis*). Table 3 displays the specific growth rate (SGR), feed conversion ratio (FCR), and survival rate with various experimental diets. We may infer from the data in Table 2 that the T_{12} treatment group experienced the most weight gain (67.31 gm), followed by the T_{13} group (63.99 gm), treatment T_{12} (415.75%) had the largest percentage of weight gain, followed by treatment T_{13} (395.48%), treatment group T_{12} had the largest net length growth (9.41 cm) followed by T_{13} (9.01 cm) group, T_{12} had the highest percentage length gain (84.77%) followed by T_{13} (82.13%). These data lead us to the conclusion that, in control laboratory conditions, the combined effect of 45 mg/kg Iron oxide nanoparticles and 1.5% concentration of the probiotic *Bacillus subtilis* (T_{12}) is more beneficial for maximal growth in *Labeo rohita*.

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

Treatment	Fish weight (g)		Net weight gain(g)	% weight gain	Fish length (cm)		Net length gain (cm)	% length gain
	Initial	Final			Initial	final		
T_1	15.96±0.43	62.08±1.15	46.12	288.97	10.76±0.35	17.21±0.48	6.45	59.94
T_{10}	16.00±0. 35	71.25±1.25	55.25	345.31	10.89±0.29	18.81±0.45	7.92	72.72
T_{11}	16.27±0.48	77.45±1.32	61.18	376.03	11.07±0.33	19.54±0.49	8.47	76.51
T_{12}	16.19±0.59	83.50±1.40	67.31	415.75	11.10±0.31	20.51±0.44	9.41	84.77
T_{13}	16.18±0.40	80.17±1.28	63.99	395.48	10.97±0.30	19.98±0.44	9.01	82.13

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

Treatment	Specific Growth Rate	Feed Conversion Ratio	Survival rate
T1PN (Control)	1.50	3.04	86.66
T_{10}	1.71	2.84	86.66
T_{11}	1.73	2.75	93.33
T_{12}	1.82	2.66	100
T_{13}	1.77	2.71	100

Information on growth parameters in the fish *Labeo rohita* can be found in Table 3. T_{12} (1.82) had the highest specific growth rate in these data, followed by T_{13} (1.77). T1PN (3.04) had the highest feed conversion ratio, followed by T10 (2.84). The highest survival rates were seen in T_{13} (100) and T_{12} (100).

Discussion

The World Health Organization (FAO) claims that probiotics improve water quality, lower mortality, and increase disease resistance. In Nile tilapia (*Oreochromis niloticus*), probiotic *Lactobacillus sp*. improves body weight, crude lipid, and total protein (Hamdan *et al*., 2016) [10]. Probiotics support energy production in the gastrointestinal tract (GIT) and aid in the digestion of nutrients in aquatic animals. Fish with excess of probiotic bacteria in the gastrointestinal tracts (GIT) experience toxicity, which can lead to organ failure or even death. So that the quantity of probiotics and nanoparticles may vary according to fish species, age and aquatic environment. (Kothari and associates, 2019) [15]. Anemia and poor development are the results of an iron deficit in fish nutrition. Iron oxide nanoparticles can help treat this iron deficiency (Thangapandiyan *et al*., 2020) [24]. According to Behra *et al*. (2014), iron oxide nanoparticles are a more accessible form of iron in fish, rats, and humans. In *Labeo rohita*, it can aid in enhancing physiological, biochemical, and hematological functions (Thangapandiyan *et al*., 2020) [24]. In this study, we examine the effects of varying probiotic concentrations (*Bacillus subtilis*) and iron oxide nanoparticles on several *Labeo rohita* fish groups. Fish fed feed with probiotics (*Bacillus subtilis*) and iron oxide nanoparticles showed a remarkable increase in length and weight.

Conclusion

The effects of Iron oxide nanoparticles and the probiotic *Bacillus subtilis* on the growth parameters of *Labeo rohita* fish have been discovered in this experimental work. After feeding with diet containing 1.5% probiotic and 45 mg/kg of iron oxide nanoparticles, experimental group T12 demonstrated the best growth performance. So that probiotics and Iron oxide nanoparticles aid in the right amounts in the fish feed could promote fish growth.

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