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Profile of water quality parameters in culture of *Labeo rohita* supplemented with *Lactobacillus sporogenes*

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Abstract
The effect of probiotic *Lactobacillus sporogenes* supplemented feed on the water quality parameters for over a period of 90 days. Indian major carp *Labeo rohita* has opted for the culture system where as the feed contained 28% protein with different dosages of *Lactobacillus sporogenes* at 10^4 CFU/g, 10^6 CFU/g, 10^8 CFU/g and control in triplicates. Uniform sized fish fingerlings averaging 0.76g were used for the experiments. The concentration of different water quality parameters viz, temperature, pH, dissolved oxygen, free carbon dioxide, total alkalinity and ammonia-nitrogen in the treated group and slightly low in the control fed group. The present study revealed that normalcy of water quality was well maintained during the culture period.

Keywords: Labeo rohita, Lactobacillus sporogenes, fish, protein

1. Introduction
The total world is 70.9% is occupied by water source and the rest 29.1% is of land area. Water body areas are characterized by natural artificial water bodies. In the world highest water spread and largest fish producing country is china which stands 1st in aquaculture production then followed by India ranks 2nd in the overall fish production of the world with a total fish production of 8.0 million tons stand 2nd in aquaculture production next to China, it also accounts for over 5.42% of the global fish production. Generally, fish culture practiced in natural ponds or in artificial water bodies needs proper water quality checks to keep fishes healthy and to get a high yield. To get these form of optimal growth and survival to yield a complete form of relationship between water quality and aquatic productivity need to be understood completely (Boyd 1982) [5]. Whereas the freshwater aquaculture in India is dominated by carps (*Labeo rohita*, *Catla catla* and *Cirrhinus mrigala*) which contribute about 87% of the total freshwater production (ICLARM, 2001) [15]. By maintaining good physicochemical properties and biological diversity in ponds represents the healthy aquatic ecosystem (Venkatesharaju *et al.*, 2010) [25]. Water quality includes all physical, chemical, and biological factors that influence aquaculture which have the main influence on survival, reproduction, growth or management of fish or other aquatic creatures.
However, poor water quality and disease out breaks are the main constraints in aquaculture production thereby affecting both economic development and socio-economic status of global production in many countries. In recent years, research on probiotics for aquatic animals is increasing with the demand for environment-friendly aquaculture practices (Dimitroglou *et al*., 2011; Iribarren *et al*., 2012) [12, 16]. The recent attempt being made to improve water quality in aquaculture by applying probiotics enzymes directly in to feed during feed preparation. Likewise, the application of different probiotic species in aquaculture is practiced to monitor water quality are Bacillus sp. (Kumar *et al.*, 2008, Lactococcus *sp* Hagi *et al.*, 2004; Sugita *et al*., 2009) [18, 13, 23], Lactobacillus *sp* (Ramakrishnan *et al.*, 2008) [20]. Hence, the present study was aimed to investigate the influence of probiotics *Lactobacillus sporogenes* on few water quality parameters in the tanks which are treated with probiotics and compare the results with those of the untreated probiotic tank.

2. Materials and Methods
2.1 Fish and experimental design
Uniform sized fingerlings of *Labeo rohita* with an average weight and length of 1.30g and

1.17cm respectively were stocked @ 15 numbers/tank. The experiment was carried out for a period of 90 days. Fecal matter and uneaten food were removed daily in the morning hours. Three test diets namely F1 10^4 CFU/g *L. sporogenes*, F2 10^6 CFU/g *L. sporogenes*, F3$10^8$ CFU/g *L. sporogenes* added to 28% protein content feed was prepared during the experimental period which was formulated using the persons square method (Hardy, 1980). Fishes were fed at the rate of 5% of their body weight till the end of the experiment. The feed was broadcasted over the surface of water twice daily in the morning and evening. After each sampling the quantity of feed given was readjusted based on the increased weight of fish.

2.2 Measurements of water quality parameters
Water quality parameters were maintained within the normal range throughout the experimental period. Water samples collected on each sampling day were analyzed for pH, temperature, dissolved oxygen, free carbon dioxide, NH_3 and total alkalinity. Digital portable kit model CK 704 was used to record pH, atmospheric temperature and water temperature. Dissolved oxygen was estimated by Winkler's method. Total alkalinity, NH_3 and free carbon dioxide were determined by following standard methods (Apha, 1995) [2].

3. Results
The results obtained are presented below.

Temperature
Table 1 shows the fluctuation in air and water temperature recorded at 15 days interval during the experimental period. The temperature of air and water ranged from 29.1 ^{0}C to 29.6 ^{0}C and 30.1 ^{0}C to 30.5 ^{0}C respectively.

pH
The pH recorded during the study period was near neutral to alkaline, ranging from a mean value of 7.56 to 8.05 in F0, 7.73 to 8.03 in F1, 7.72 to 7.95 in F2 and 7.72 to 8.05 in F3 (Table 2).

Dissolved oxygen
The values of dissolved oxygen are recorded on different sampling days are presented in Table 3. The average values of dissolved oxygen were 7.33 to 7.76 mgl^{-1} in F0, 7.53 to 7.81 mgl^{-1} in F1, 7.34 to 7.72 mgl^{-1} in F2 and 7.49 to 7.86 mgl^{-1} in F3.

Free carbon dioxide
The values of free carbon dioxide recorded over the experimental period are shown in Table 4. The average values of free carbon dioxide were 1.10 to 2.11 mgl^{-1} in F0, 1.14 to 2.03 mgl^{-1} in F1, 1.15 to 2.19 mgl^{-1} in F2, 1.10 to 2.00 mgl^{-1} in F3.

Total alkalinity
The total alkalinity values recorded in the different tanks during the experimental period are presented in Table 5. The total alkalinity value recorded during the experiment period was 52.66 to 109.33 mgl-1 of CaCO3 in treatment F0, 58.00 to 117.33 mgl-1 of CaCO3 in treatment F1, 56.00 to 115.66 mgl-1 of CaCO3 in treatment F2, 61.00 to 105.66 mgl-1 of CaCO3 in treatment F3.

Ammonia-Nitrogen
The ammonia-nitrogen estimated during the experimental period is presented in Table 6. The average values of ammonia-nitrogen ranged from 0.039 to 0.313µg $^{l-1}$in F0, 0.034 to 0.293 µg $^{l-1}$ in F1, 0.045 to 0.310 µg $^{l-1}$in F2 and 0.041 to 0.275 µg $^{l-1}$ in F0.

Table 1: Air and water temperatures (°C) recorded during different sampling days of the experimental period.

Days after stocking	Air temperature (°C)	Water temperature (°C)	Difference (°C)
0	30.1	29.2	0.9
15	30.1	29.1	1
30	30.2	29.4	0.8
45	30.4	29.6	0.8
60	30.5	29.4	1.1
75	30.4	29.4	1
90	30.2	29.2	1

Table 2: pH of water recorded during different sampling days of the experimental period

Treatments	Replications	No. of days						
		0	15	30	45	60	75	90
F0	Mean	8.05	7.56	7.66	7.78	7.82	7.71	7.52
	SE	0.008	0.072	0.073	0.107	0.064	0.11	0.145
F1	Mean	8.03	7.74	7.7	7.73	7.8	7.87	7.73
	SE	0.006	0.006	0.008	0.008	0.008	0.012	0.02
F2	Mean	7.95	7.7	7.76	7.72	7.82	7.77	7.64
	SE	0.076	0.008	0.012	0.014	0.014	0.013	0.014
F3	Mean	8.05	7.68	7.62	7.72	7.86	7.72	7.4
	SE	0.008	0.049	0.028	0.014	0.005	0.028	0.053

Table 3: Profile of dissolved oxygen (mg l^{-1}) of water recorded during different sampling days of the experimental period.

Treatments	Replications	No. of days						
		0	15	30	45	60	75	90
F0	Mean	7.66	7.33	7.60	7.53	7.76	7.67	7.33
	SE	0.031	0.103	0.026	0.020	.0145	.0145	0.008
F1	Mean	7.53	7.56	7.68	7.66	7.81	7.72	7.47
	SE	0.023	0.012	0.015	0.011	0.012	0.008	0.008
F2	Mean	7.34	7.48	7.48	7.72	7.69	7.71	7.51
	SE	0.178	0.039	0.032	0.008	0.032	0.034	0.020
F3	Mean	7.64	7.55	7.68	7.79	7.86	7.75	7.49
	SE	0.020	0.063	0.008	0.030	0.017	0.012	0.011

Table 4: Free carbon dioxide profile of water (mg l^{-1}) recorded during different sampling days of the experimental period

Treatments	Replications	No. of days						
		0	15	30	45	60	75	90
F0	Mean	1.10	1.44	2.07	1.97	1.96	1.94	2.11
	SE	0.006	0.053	0.135	0.102	0.204	0.243	0.118
F1	Mean	1.14	1.51	1.72	2.00	1.93	1.24	2.03
	SE	1.15	1.79	2.01	2.35	1.77	1.61	2.19
F2	Mean	0.005	0.029	0.156	0.066	0.205	0.037	0.141
	SE	0.06	0.02	0.08	0.06	0.12	0.03	0.28
F3	Mean	1.10	2.00	1.97	1.91	1.98	1.54	2.24
	SE	0.017	0.061	0.0753	0.081	0.200	0.046	0.077

Table 5: Total alkalinity Profile of water (mg l^{-1}) recorded during different sampling days of the experimental period

Treatments	Replications	No. of days						
		0	15	30	45	60	75	90
F0	Mean	65.66	52.66	75.66	103.66	107.66	111	109.33
	SE	0.05	0.666	0.333	0.881	1.452	3.214	1.210
F1	Mean	62.33	58.00	76	92.33	102.66	117.33	113.66
	SE	4.37	1.527	1.154	0.881	1.452	1.452	0.881
F2	Mean	67.33	56.00	66.66	102.66	100.66	111.66	115.66
	SE	3.52	3.05	3.17	1.45	0.66	1.20	0.88
F3	Mean	75.33	61.00	63.33	102.00	97.66	105.33	105.66
	SE	3.17	1.527	0.881	0.577	1.45	2.72	1.210

Table 6: Ammonia-Nitrogen (µg l^{-1}) of water recorded during different sampling days of the experimental period.

Treatments	Replications	No. of days						
		0	15	30	45	60	75	90
F_0	Mean	0.039	0.134	0.184	0.220	0.313	0.287	0.260
	SE	0.003	0.005	0.007	0.005	0.0355	0.008	0.130
F1	Mean	0.034	0.150	0.177	0.243	0.284	0.293	0.265
	SE	0.003	0.002	0.005	0.006	0.004	0.004	0.010
F2	Mean	0.045	0.150	0.191	0.230	0.252	0.310	0.284
	SE	0.003	0.004	0.003	0.012	0.005	0.003	0.004
F3	Mean	0.041	0.159	0.172	0.245	0.271	0.275	0.251
	SE	0.002	0.004	0.004	0.009	0.004	0.006	0.007

4. Discussion

The quality of water is very important in the culture of *L. rohita*, since the use of feeds is known to have an influence on water quality which directly affecting the species cultured. Where water quality is simply defined as the degree of excellence that given water possesses for the propagation of desirable aquatic organisms to achieve high survival, growth and reduction (Deo, 2006) [11]. Maintenance of good water quality with an adequate level of oxygen which is essential for metabolism activity, survival and optimal growth of *L. rohita.* A complete understanding of the relationship between water quality and aquatic productivity is a pre-requisite for optimum growth and survival (Boyd, 1982) [5]. In the present study, important water quality parameters such as temperature, pH, dissolved oxygen, free carbon dioxide, total alkalinity and ammonia-nitrogen were measured throughout the experimental period. The water quality parameters measured in different treatments thought the experimental period was found to be well within the acceptable range for *L. rohita* culture.

Temperature is a major factor, which directly influences metabolism affecting all physiological processes in ectotherms such as food intake, metabolism and nutritional efficiency (Brett, 1979; Burel *et al*., 1996) [6, 7]. Thus, water temperature directly affects the growth of fish (Smith, 1989) [22]. Therefore, knowledge of suitable temperatures at which fish have a faster growth rate is very important for the effective management of aquaculture systems (Cui and Wootton, 1988) [9]. Few research article recorded the temperature at 28 ^{0}C which improved the growth and survival of *L. rohita*. However, carps are reported to thrive well between 18 ^{0}C to 37 ^{0}C (Jhingran, 1975) [17]. Water and air temperature recorded during the present study ranged from 29.1 ^{0}C to 29.6 ^{0}C and 30.1 ^{0}C to 30.5 ^{0}C respectively, which is considered to be within the tolerance limit of *L. rohita*.

The pH of water is a measure of hydrogen ion concentration and indicates whether the water is acidic or basic. The water has a pH range of 6.5-9.0 is more suitable for fish culture and values above 9.5 are unsuitable as carbon dioxide becomes unavailable at higher pH (Das *et al*., 1995) [10]. Fish dies at a pH of above 11.0. Acidic waters reduce the appetite and retard the growth. Das *et al*. (1995) [10] suggested that a pH range of 6.12-8.6 is most suitable for the survival of the Indian major carp fry. Neutral to slightly alkaline pH has been found to be most favorable for fish ponds (Swingle, 1961; Banerjee, 1967) [24, 3]. While correlating productivity of a large number of ponds having different soils and water properties is affected and near neutral to slightly alkaline pH range found to be the most favorable for fish ponds. The pH in the present study ranged between 7.3 and 8.07, which is in the desirable limits for the optimum growth of *L. rohita.* Whereas the pH ranges for diverse fish production is between 6.5 and 9 (Boyd and Tucker, 1998) [4].

Dissolved oxygen is the most important environmental factor influencing the health condition of fish in aquaculture Systems because must need an element of life is oxygen. An adequate amount of oxygen helps in water helps in providing better metabolism in fishes and helps in the survival of

aerobic life form. Among the dissolved gases, the dissolved oxygen plays the most important role with regard to the water quality. According to Banerjee (1967) [3], cyprinids require 6-7 ppm oxygen for good growth, but they can tolerate levels as low as 3 ppm for short periods. DO values higher than 5 mg l^{-1} have often been recommended for intensive culture practices (Cheng *et al*., 2003) [8]. Banerjee (1967) [3] reported that oxygen concentration above 5ppm is indicative of productivity, but dissolved oxygen below that level indicates that the water is unproductive. The dissolved oxygen content observed in the present study was 7.22 to 7.9 mg l^{-1}. It is an unacceptable limit and suitable for the optimum growth of *L. rohita*.

Carbon dioxide in a gaseous state which is present in the atmosphere in very small quantity. The main source of carbon dioxide in pond water is through absorption from the atmosphere, decomposition of organic matter and respiration of aquatic animals. Aquatic animals are affected by carbon dioxide depending on the oxygen level of water. If there is sufficient oxygen in pond water fish can survive at carbon dioxide levels as high as 60 ppm (Hart, 1944). It has been reported that carbon dioxide provides the inorganic carbon essential for photosynthesis, thereby being a decisive factor in organic production (Swingle, 1961) [24]. The effect of CO_2 on aquatic animals is dependent on oxygen concentration in water. CO_2 concentrations in intensively managed aquaculture waters normally fluctuate between 0 to > 20 ppm free CO_2 in a 24-hour cycle with the lowest concentrations during the hours of photosynthesis (Schmittou, 1998) [21]. The recorded free carbon dioxide in the present study ranged from 0.94 to 2.98 mg l^{-1} and considered as acceptable for optimum growth of *L. rohita*.

Total alkalinity is the total concentration of bases in water expressed in mg/l of equivalent calcium carbonate (Boyd, 1982) [5]. It refers to the buffering capacity of water. Natural water that contains 40 mg/l or more total alkalinity considers more productive than waters of lower alkalinity (Moyele, 1945) [19]. The alkalinities in the range of 20-120mg/l have a little effect on fish production (Banerjee, 1967) [3]. Ponds with alkalinity greater than 300 mg l^{-1} may be unproductive because of limitations to carbon dioxide availability at such high concentrations (Adhikari, 2000) [1]. Boyd (1982) [5] total alkalinity value range between 20 to 300 mg l^{-1} is ideal for fish and less than 20 mg l^{-1} alkalinity creates stress in fish. The total alkalinity values in this study fluctuated between 52 to 120 mg l^{-1} and it was in the acceptable range for the culture of *L. rohita*.

The source of ammonia-nitrogen in water is excreta of cultured animals and microbial decay of nitrogenous compounds. The major end product of protein catabolism is ammonia which is excreted primarily as un-ionized ammonia by fish. Nitrogenous substances present in the faces and uneaten feed reach water and get mineralized rapidly releasing ammonia in-to the water. Wang *et al*. (2005) [26] also showed that probiotics could reduce the concentrations of nitrogen and phosphorus in pond water as compared to the control supporting the present results and advocating the use of In the present study, the levels of ammonia-nitrogen were insignificant in the first two sampling in all treatment and control tanks and gradual build-up was observed in subsequent samplings due to increasing in feeding rate. The ammonia values recorded during the present investigation were ranged from 0.029 to 0.384 mg l^{-1} and it was below the tolerance limit of the carps (Das *et al*., 2004) [10].

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