Pak. J. Agri. Sci., Vol. 59(1), 135-145; 2022
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/22.660
http://www.pakjas.com.pk

Effect of probiotics on growth and health status of *Labeo rohita*

Kinza Asghar[1], Fayyaz Rasool[1,*], Shakeela Parveen[2], Muhammad Akmal[1], Khalid Mahmood Anjum[3], Matiullah[1] and Shahid Mahmood[1]

[1]Department of Fisheries and Aquaculture, Faculty of Fisheries & Wildlife, University of Veterinary and Animal Sciences Lahore, Pakistan; [2]Department of Zoology, Wildlife & Fisheries, Faculty of Sciences, University of Agriculture, Faisalabad, Pakistan; [3]Department of Wildlife and Ecology, Faculty of Fisheries & Wildlife, University of Veterinary and Animal Sciences Lahore, Pakistan
[*]Corresponding author's e-mail: fayyazrasool@uvas.edu.pk; drfayyaz1980@gmail.com

A feeding trial of 90 days was conducted to check the effect of probiotic on the growth of *Labeo rohita*. Four different doses of feed T1 (0.5×107), T2 (1 ×107), T3 (1.5×107) and control were formulated. Fish were fed at the @3% body weight, twice a day total of 300 *L. rohita* having an average weight of 10-15 g were collected from the University of Veterinary and Animal Sciences Ravi Campus, Pattoki (UVAS) fish ponds. Total 12 aquaria were used and 25 fish in each aquarium were stocked such as control, T1, T2 and T3. Initial weight and length of each fish were recorded before stocking. Water quality parameters were checked daily. On weekly basis weight and length was examined, at the end of the experimental trial; the final weight and length were resulted. Treatment T2 (57.11±0.51) growth parameters were recorded significantly, while T3 (42.28±0.35) and T1 (48.70±0.06) were recorded non-significant. Treatment T1 was a significant result of hematological parameters while T2 and T3 were recorded non-significantly. In all treated groups, the physico-chemical parameters were remained statistically non-significant throughout the experimental trial.
Keywords: Weight gain, FCR, SGR, body composition, hematology.

INTRODUCTION

Aquaculture is a major source to enhance the demand of animal and plant protein, and hopeful enterprises for supporting human nutrition and food security ((Panigrahi and Azad, 2007). Aquaculture is a part of agriculture, has a main food chain awareness and its main purpose is to make food production sustainable (Panigrahi and Azad, 2007). The average use of the aquaculture items has improved up to 47% in 2006 which was 14% in 1986 and a 50% further increase is estimated in coming years (Mohapatra *et al*., 2013). In 2018 the international global export share of developing countries increased to 54 % and total volumes up to 60% (FAOSTAT, 2020). Fish are the main sources of protein for humans and also utilized as a bio-indicator in a biome (Schon *et al.,* 2006). Fishes have a high level of protein and vitamins (A, B, D, E), minerals (calcium, phosphorus, iron, zinc and iodine) and a low quantity of fats (Roos *et al.,* 2007). In economical and industrial scale fish have high level of production gradually. The high value and most suitable fishes are cultured in the fish farming systems in many countries but in some countries, aquaculture system is facing main hindrance of different disease outbreak, poor growth and low survival of fish due to harmful pathogens (virus, fungi, bacteria, protozoa and parasites) with the unhygienic aquatic environment can be source of decrease in this supply chain (Sakai, 1999). Pathogenic bacteria and virus cause high mortality and huge economic losses in aquaculture industry (Wang *et al.,* 2008). To control the high tiding of pathogenic bacteria and viruses used of antibiotic and vaccine in aquaculture practices (Robertson *et al.,* 2000; Wang *et al.,* 2005). Different studies revealed that excess and misuses of antibiotics causes antibiotic resistance. The emerging practices in aquaculture uses of probiotics to control the outbreak of pathogenic bacteria. The probiotics uses in aquaculture has not showed only the reduction of dangerous anti-microbial chemicals, particularly some antibiotics, but also develop appetite and bio-growth performance of the farmed species in an eco-friendly and maintainable manner (Robertson *et al.,* 2000; Wang *et al.,* 2005).

Probiotics are microbes which help to protect the organism from the disease. According to Fuller (1992) probiotics are feed supplements which can improve the balance of intestinal microbes. Microbes show high significant and curial impact

Asghar, K., F.Rasool, S. Parveen, M. Akmal, K. M. Anjum, Matiullah and S. Mahmood. 2022. Efficacy of subsurface and surface drip irrigation regarding ater productivity and yield of maize**.** Pakistan. Journal of Agricultural Sciences.59:135-145.
[Received 24 May 2021; Accepted 23 Nov 2021; Published 18 Mar 2022]

on aquaculture; because of water quality parameter and control of disease are affected by activities of microbes. Probiotics can enhance nutritional competition and antibacterial substances production and considered as highly valuable for the fish diseases control. In aquaculture, constant problems of disease demand the bacterial use as probiotics and as alternative to antibiotics (Irianto and Austin, 2002).

The results of the trials determined that probiotics utilized as antibiotics has ability to improve fish growth (Kumar *et al.,* 2006). It has been observed in trial mixed three different species like; *Bacillus subtilis, Lactococcus lactis*and *Saccharomyces cerevisiae*in the *L. rohita* diet to enhanced production of digestive enzyme and nutrient utilization of fish.

There are many strains of microbes used as antibiotics in aquaculture. The common antibiotics utilize in aquaculture, belong to *Enterococcus* spp., *Bicdobacterium* spp., *Bacillus* spp, *Saccharomyces* spp, *Vibrio* spp, *Lactobacillus* spp, and *B. subtilis* are used in aquaculture for bacteriotherapy. Intake of suitable amount of *B. subtilis* is expected to revive the normal microflora after use of antibiotics (Green *et al.,* 1999). The probiotic strains used in the feed of grow out fish, to enhance the parameters of development (Ghosh *et al.,* 2002). The appropriate probiotics used in the industry of aquaculture showed improvement in intestinal microbial balance and absorption of feed, thus leading to improved growth rate (Rengpipat *et al.,* 1998) and reduced FCR (feed conversion ratio) during the cultural period (Wang *et al.,* 2005). *Bacillus* species have been used in fish feed to improve the feed utilization, growth rate and control the bacterial infection (Kavitha *et al*., 2018; Ramesh and Souissi, 2018)

The *B. subtilis* colonization in the gut epithelium decreases risk of infection of pathogenic bacteria and increase the capacity of fish to save themselves from certain diseases. It had comparably higher in fish that fed on diets containing *S. cervisiae*. Significantly increases in the training and enterprises council (TEC) during pre-challenge in fish that fed on *B. subtilis* are an indication of enhanced growth and health of the fish (Duncan and Klesius, 1996).

Keeping in view the importance of probiotics supplementation in fish feed utilization and their impacts on enzyme activity, the present study was planned to determine the effect of *B. subtilis,* on growth performance, proximate composition and hematology of *L. rohita.*

MATERIALS AND METHODS

***Experimental Design, Feed Formulation and Feeding*:** The experimental trial was conducted in the Fish Seed Rearing Facility in glass aquaria (30" x 12" x 18"), University of Veterinary and Animal Sciences, Ravi, Pattoki (UVAS). Total 300 *L. rohita* having average weights of 10-15 g were collected from fish ponds of UVAS. Total 12 aquariums were used and 25 fish were stocked in each aquarium such as control, T1, T2 and T3. *Bacillus subtilis* strain was obtained from Mycology Laboratory of Government College University (GCU), Lahore, and other feed ingredients fish oil, fish meal, soya bean meal, grain by product, plant protein cereal and vitamins premix were purchased from local market. Feed proximate composition were; crude protein (30%), ash (8%), crude fat (6%), moisture (9%) and crude fiber (5%). Fish feed was prepared by different ratio. CP level of basal diet was 30% in control. Further this basal diet was added in other treatment groups in three different probiotic (*Bacillus subtilis*) levels (0.5×10^7 CFU/g, 1×10^7 CFU/g and 1.5×10^7 CFU/g) listed in Table 1. Fish were fed at the rate of 3% body weight, twice a day.

***Microbial Culture*:** Culture of *Bacillus subtilis,* serial dilution technique was applied with different solutions (10^{-1} to 10^{-4}) use of phosphate buffered saline (PBS) with pH 7.2. Each dilution was striped on Tryptic soya agar (TSA), Eosin methylene blue (EMB), Nutrient agar (NA) and Milk agar (Cazorla *et al.,* 2007: Foysal and Lisa, 2018).

Table 1. Ingredient and weight of experimental diet with four different inclusion levels *Bacillus subtilis* a probiotic.

Ingredients	Control 100g	Treatment 1 (0.5%) 100g	Treatment 2 (1%) 100g	Treatment 3 (1.5%) 100g
Crude Protein (%)	30	30	30	30
Bacillus subtilis (Probiotic, C.F.U/ml)	0.0	0.5×10^7	1.0×10^7	1.5×10^7
Fish Meal (g)	20	20	20	20
Rice Polish (g)	35	35	35	35
Soya Bean (g)	20	20	20	20
Corn Gluten (g)	23	23	23	23
Vitamin Premix (g)	1	1	1	1
Molasses (g)	1	1	1	1

Table 2. Biochemical identification of *Bacillus subtilis*

Isolate	Source	Indole	VP test	Citrate test
Bacillus subtilis	Fish gut	-	+	-

***Biochemical identification of Bacillus subtilis*:** *Bacillus subtilis* was recognized with different biochemical test; Indole, VP test, and Citrate test as previously described by (Wulff *et al.,* 2002; Foysal and Lisa, 2018) listed in Table 2.

***Physico-chemical parameters*:** Physico-chemical parameters were recorded on daily basis as previously described by Matiullah *et al.,* (2016).

***Determination of growth performance*:** During the experimental trial, weight gain (WG %), specific growth rate (SGR %), and feed conversion ratio (FCR) were calculated. Growth parameters were checked weekly. At the end of experimental trail after 90 days 10 fish were collected from each aquarium and growth performances was evaluated.

***Growth Performance*:** During the initiations of experimental trial, the initial weight was recorded and after 7 days interval weight was also measured before analysis. The following standard formulas were used for the determinations of feed efficiency and growth of fish (Hopkins, 1992)

***Absolute weight Gain (g)*:** Estimated by the formula given below

Weight gain % = (final body weight (g) – initial body weight (g)

***Feed Conversion Ratio*:** The FCR was determined by:

FCR = total dry feed intake (g)/wet weight gain

Specific Growth Rate (SGR):

Determined by the formula:

Specific growth rate = In (final body weight (g) – initial body weight (g)/ (no of days) × 100

***Proximate Analysis*:** Proximate analysis such as crude protein, crude fat, crude ash and moisture were checked at the end of trial (AOAC, 2006; Siani *et al.,* 2014). After feeding trial, fish were starved for one day, and anesthized by using 3000 mg/l clove oil for 40-60 s and final weight was measured . 4-5 fish were taken from each treatment killed and placed at 105°C in hot air oven for 24 hours. After oven drying the samples were grounded in mortar and pestle and fine powder was formed. The powdered samples (whole body and feed) were weighed and stored in polythene bags at room temperature for further analysis. Chemical compositions of the samples were determined in the fish nutrition laboratory of Fisheries and Aquaculture Department, University of Veterinary and Animal Sciences, Ravi campus, Pattoki.

***Estimation of Crude moisture % or Dry Matter*:** The crude moisture or dry matter was influenced by the method of drying in oven. One gram of whole body and diet sample (W1) were weighed in petridish. The petridish containing samples were placed in hot air oven/ Dry oven at temperature of 105°C for 12 hrs. In desiccators the samples of whole body and feed were placed and weighed after 10 minutes of cooling. Then place the dried samples again in oven for 2 hours till the weight of the samples were kept constant. Final weight (W2) of samples was noted and further evaluates the moisture % and dry matter by using following formulas:

The crude moisture % was calculated from below formula:

$$Moisture\ \% = \frac{W1 - W2}{weight\ of\ sample} \times 100$$

Whereas, Petri dish weight + Sample weight before drying = W1, Sample weight after drying Petri dish weight = W2, The formula used for the determination of dry matter was as follows:

$$Dry\ matter = 100 - moisture\ \%$$

***Estimation of Crude protein (CP) %*:** The CP % from the samples of whole body and feed were calculated from the method of micro Kjeldahl's apparatus. The samples were digested in digestion mixture (CuSO4: FeSO4: K2SO4 in 7: 3: 90). The digested mixture was cooled and dilute in distilled water. Further 10 ml of dilution was distilled with 40 % NaOH, 2 % Boric acid and methyl red indicator.

For digestion process, the samples were digested in digestion mixture (5g) and concentrated sulphuric acid (30ml) which were placed in Kjeldahl's flask. The samples containing solutions were heated on hot plate until the solution turned clear greenish in color. The digested material was placed to cool and dilute with 250 ml distilled water in measuring flask. Take 10 ml of diluted sample in Kjeldahl's apparatus.

Then 40% NaOH (10ml) was also added in the apparatus and steam distilled. Ammonia was released the distillation process completed. The ammonia was collected with the help of 10ml of solution of Boric acid (2%) by using indicator (methyl red). Ammonia was accumulated for 2 minutes until indicator color was converted to golden yellow from pink.

The titration process was done by titrating the sample with 0.1 N Sulphuric acid. From the titrated samples the nitrogen % was estimated from the formulas below:

$$\text{Percentage of Nitrogen} = \frac{\text{volume of } H_2SO_4 \text{ used} \times \text{Normality of } H_2SO_4 \times 0.014 \times 250}{\text{Sample weight}} \times 100$$

Whereas: Standard volume of 0.1 N H_2SO_4 used to neutralized 1 ml of ammonia =0.014, Dilution of digestion mixture= 250, Nitrogen %= 100, Volume of diluted sample and digestion used = 10

The formula for the protein % was as follows:

$$\text{Crude protein (\%)} = N_2 \times 6.25$$

***Estimation of Crude fat (CF) % extraction*:** The CF% was measured from whole body and feed samples using Soxhlet apparatus (Sr. no. 70861, Germany) by the method of petroleum ether extraction. The feed and whole-body sample (1g) were measured and folded in filter paper. Place the folded sample in the thimble and the condenser was fixed. The extraction cup was weighed by pressing down the heating handle. The petroleum ether or n- hexane was inserted in it up to 50-70 ml. Turn on the water tap and main switch. In the condenser the cup used for extraction was maintained. During extraction process of 15 minutes the mode knob moved to the position of "boiling" and the thimble was submerged in solvent. Hence, it was assured that the condenser valves were not closed and mode knob was influenced to the position of

"Rinsing" position for approximately 20 mints and above this the thimble containing solvent was suspended. The condenser valves were held close during the process of rinsing. Then condenser valves were opened and the release the extraction cup. A little amount of solvent (Ether or n- hexane) and fat present in the extraction cup was assigned to drying in oven. After the process of drying, weighed the sample, placed the extraction cup in desiccators for about 10 mints. The fat %was determined as follows:

$$\text{Crude Fat \%} = \frac{\text{W1} - \text{W2}}{\text{W1}}$$

Where: Weight of sample (g)= W1, Weight of extraction cup empty (g) = W2, Weight of residue (g) + extraction cup= W3

***Estimation of Crude Ash (CA) %*:** The crude ash (%) was estimated from the samples (feed and whole body) by using muffle furnace. The empty crucibles were taken and weighed them (W1). Add one-gram samples of each feed and whole body in the crucibles and weighed (W2) after that the crucibles were placed for 2-4 hours at temperature 600°C in furnace for ash content. After 2-4 hours the samples were completely oxidized. The white or grey in color ash content were obtained. Then crucibles were cooled in desiccators. After the process of cooling, the ash samples were weighed (W3). The crude ash % from the samples was determined by the formula shown below:

$$\text{Crude ash (\%)} = \frac{\text{Ash weight}}{\text{Sample weight (W2)}} \times 100$$

Whereas:

$$Ash\ weight = W3 - W1$$

***Hematological and liver function Analysis*:** The samples of fish were taken from each treatment to check the effect of probiotic on blood and liver profile (Nayak *et al.,* 2007; Rajesh *et al.,* 2008)

***Respiratory burst activity*:** Total 50 microliter (µL) of blood was place in microtiter plate and incubates for one hour at 37°C for cell adhesion. Removed supernatant and adhere cell was washed with PBS. After this added 0.2% nitrobluetetrazolium (NBT) and incubate for 1 hour. The cell was wash with 30% methanol and 60µL potassium hydroxide and 70 µl dimethyl sulphoxide was added and dissolve. The precipitate of formazan blue was observed (Stasiack *et al.*, 1996)

***Statistical analysis*:** One-way ANOVA was applied on collected data of various parameters. Comparison of means was checked by Duncan's Multiple Range Test (Steel *et al.*, 1996).

RESULTS

During the research trial, *L. rohita* was grown under controlled conditions with three different feeding strategies viz. Treatment 1 with 0.5%, Treatment 2 with 1%, Treatment 3 with 1.5% probiotic levels and control group without probiotic, for a period of 90 days. There were no statistical differences at the start of the trial between control and experimental groups; initial average weight was similar indicating that the fish health and condition was similar. After 90 days of trial, data related to growth parameters, hematological parameters, proximate analysis and physicochemical parameters were recorded and analyzed for statistical differences.

***Fish Growth Responses*:** Growth parameters, such as increase in average wet weight (g), increase in average fork length (mm), increase in average total length (mm), feed intake (g), feed conversion ratio (FCR), condition factor (K) and survival rate were recorded every fortnight. Highest average increase in wet weight was recorded in treatment 2 as 57.11±0.51among all treatments while lowest recorded 39.24±0.33 in control group. Average increase in fork length was highest in treatment 2 65.06±0.25 among all treatments while lowest 25.92±0.61 in treatment 3. Highest value of average feed intake observed 140.74±1.23 in treatment 1 while lowest 67.79±0.65 feed intake recorded in control group listed in Table 3.

***Hematological parameters and Liver function test*:** Blood samples of fish was collected from each treatment with the help of syringes in EDTA (anticoagulant) *vacutainer* tube, and the different parameters were calculated for each treatment: TLC (total leukocyte count), neutrophil, lymphocytes, monocytes, eosinophil, platelets, total RBC, hemoglobin, while in liver function test total proteins, albumins and globulins were calculated. Concentration of TLC and RBC were calculated higher in treatment 3 among all treatments as 5.1×10^5/µL and 4.10×10^6/µL. Concentration of the number of Neutrophil were highest in treatment 3 with

Table 3. Overall Growth Responses of *L. rohita* Fed with and without probiotic added feed

Treatments	Average increase in wet weight (g)	Average increase in fork length (mm)	Average increase in total length (mm)	Average feed intake (g)	Feed conversion ratio	Condition factor (K)
Treatment 1	48.70±0.06^b	38.96±0.11^c	45.50±0.63^b	140.74±1.23^a	2.95±0.01^a	2.87±0.01^a
Treatment 2	57.11±0.51^a	65.06±0.25^a	53.01±0.02^a	93.26±0.98^b	1.66±0.01^d	2.05±0.14^d
Treatment 3	42.28±0.35^c	25.92±0.61^d	25.67±0.40^c	91.63±0.85^b	2.21±0.01^b	2.55±0.03^b
Control fish	39.24±0.33^c	56.68±0.42^b	47.16±0.55^b	67.79±0.65^c	1.77±0.01^c	2.22±0.04^c

Means with same letters in a single column are statistically similar at $p< 0.05$.

86% while lowest as 80% recorded in control group, lymphocytes were recorded highest in treatment 2 and 3 with equal value of 8% while lowest values were recorded for treatment 1 with 5.90%, monocytes were calculated with highest concentration of 3.70% in treatment 2 while lowest in treatment 1 with 2.90%, eosinophil were recorded 3% with highest value in control group while 2.10% the lowest in treatment 1. Highest concentration of hemoglobin was observed in treatment 3 and its value was 11.30/µl while the lowest value 7.21/µl was recorded in treatment 1. Platelets were recorded among all treatments and the highest values were observed as 1.69×10^5 in treatment 3 while lowest numbers of platelets were recorded 1.52×10^5 in control group. Liver function test was also performed for all the treatments to record total proteins, albumins and globulins. The total proteins recorded among all treatments the highest value was recorded 8.50 g/L in treatment 2 while the lowest value was observed 6.80g/L in control group. Albumin was calculated highest 5.90g/L in treatment 2 while lowest value observed in treatment 1 with 4.10g/L. The highest and lowest concentration of globulin were recorded as 2.80g/L and 1.90g/L for treatment 1 and control group respectively Table 4.

***Proximate Analysis*:** Body composition of *L. rohita* was done at the end of the 12 weeks experimental period and 3 fish from each treatment were collected and sacrificed for the analysis, the values of parameters viz. crude protein, crude fat, moisture, dry matter, ash, carbohydrates and crude fiber were calculated and presented in Table 5. Percent values of moisture contents were observed as 0.35%, 0.64%, 0.17% and 1.5% among the treatments 1, 2, 3 and control group in which the highest value was recorded in control group, while treatment 3 showed the lowest value. Concentration of dry matter in percent was recorded for treatment 1, 2, 3 and control group were 99.65%, 99.36%, 99.82% and 99.98% respectively. Values for the crude fat during proximate analysis was calculated as 9.17%, 8.53%, 8.59 and 6.50% respectively for the treatment 1, 2, 3 and control group in which the highest value was 9.17% in treatment 1, while lowest value was recorded 6.50% in control group. The values of Ash were observed in treatment 1, 2, 3 and control group as 15.90%, 16.21%, 9.53% and 17.00%. The calculated values of fiber were recorded as 0.37%, 0.20%, 0.15% and 0.24% respectively in treatment 1, 2, 3 and control group. The percent values of crude protein were calculated during proximate analysis as 70.18%, 61.25%, 65.84% and 57.00% accordingly in treatment 1, 2, 3 and control group. Carbohydrates were calculated by the summation of all the above parameter values and its subtraction from 100. The resulting values were recorded as 8.83%, 9.35%, 4.63% and 0.55% in treatment 1, 2, 3 and control group, respectively.

***Physico-chemical parameters*:** During the 12 weeks experimental trial, physicochemical parameters were recorded on daily basis and the 6 fortnightly mean values of all the parameters viz. temperature, pH, total hardness, total ammonia, dissolved oxygen, electrical conductivity, Total Hardness, Calcium and Magnesium were calculated separately for each treatment. All physico-chemical parameters were recorded non-significant for treatment 1. The mean value recorded for temperature was 30.10±0.26ºC. pH was maintained throughout the experiment and the mean value was recorded as 7.03±0.11. The total hardness recorded

Table 4. Hematology Parameters

	Units	Treatment 1	Treatment 2	Treatment 3	Control
TLC	/µL	4.7×10^4	4.9×10^4	5.1×10^5	4.6×10^4
Total RBC	/µL	2.33×10^6	3.00×10^6	4.10×10^6	2.52×10^6
Haemoglubin	/µl	7.21	9.85	11.30	6.40
Platlets	/µl	1.53×10^5	1.68×10^5	1.69×10^5	1.52×10^5
Neutrophil	%	84.10	85.00	86.00	80.00
Lymphocytes	%	5.90	8.00	8.00	6.00
Monocytes	%	2.90	3.70	3.60	3.00
Eosinophils	%	2.10	2.30	2.40	3.00
Total protein	g/L	6.90	8.50	7.30	6.80
Albumin	g/L	4.10	5.90	5.10	4.90
Globulin	g/L	2.80	2.60	2.20	1.90

Table 5. Proximate composition of *L. rohita* fed with and without probiotic added feed

Treatments	Moisture (%)	Dry matter (%)	Fat (%)	Ash (%)	Fiber (%)	Protein (%)	Carbohydrates (%)
Treatment 1	0.35 ± 0.05^b	99.65 ± 1.23^a	9.17 ± 0.65^a	15.90 ± 1.12^a	0.37 ± 0.05^a	70.18 ± 2.35^a	8.83 ± 0.23^a
Treatment 2	0.64 ± 0.06^a	99.36 ± 2.25^a	8.53 ± 0.25^{ab}	16.21 ± 1.31^a	0.20 ± 0.09^b	$61.25\pm2.16a^b$	9.35 ± 0.21^a
Treatment 3	0.17 ± 0.05^c	99.82 ± 1.98^a	8.59 ± 0.36^{ab}	9.53 ± 0.09^b	0.15 ± 0.02^c	$65.84\pm1.65a^b$	4.63 ± 0.32^b
Control fish	0.02 ± 0.01^d	99.98 ± 2.65^a	6.50 ± 0.05^c	17.00 ± 1.11^a	0.24 ± 0.06^b	57.00 ± 3.02^b	0.55 ± 0.15^c

Means with same letters in a single column are statistically similar at $p< 0.05$.

Table 6. Average Physico-chemistry of *L. rohita* test medium among all treatments.

	Temperature (ºC)	pH	Total Hardness (mgL^{-1})	Total Ammonia (mgL^{-1})	Dissolved Oxygen (mgL^{-1})	Electrical conductivity ($mScm^{-1}$)	Calcium (mgL^{-1})	Magnesium (mgL^{-1})
Treatment1	30.10±0.26[a]	7.03±0.11[a]	200.65±1.30[a]	0.43±0.30[a]	6.79±0.29[a]	2.92±0.18[a]	15.33±0.36[a]	40.18±0.33[a]
Treatment2	30.01±0.33[a]	7.07±0.10[a]	199.67±1.33[a]	0.51±0.18[a]	6.51±0.24[a]	2.66±0.08[a]	15.26±0.41[a]	40.26±0.40[a]
Treatment3	29.98±0.22[a]	7.01±0.12[a]	199.95±1.29[a]	0.76±0.25[a]	6.70±0.38[a]	2.60±0.10[a]	14.83±0.57[a]	40.31±0.52[a]
Control	29.90±0.23[a]	7.03±0.11[a]	199.61±0.97[a]	0.46±0.13[a]	6.28±0.14[a]	2.54±0.12[a]	15.10±0.37[a]	40.26±0.36[a]
Means±SD	29.99±0.08	7.07±0.02	199.99±0.47	0.54±0.15	6.57±0.22	2.68±0.16	15.13±0.22	40.25±0.05

Means with same letters in a single column are statistically similar at $p< 0.05$.

during 6 fortnights was non-significant and the mean value was 200.65±1.30 mgL^{-1}. The mean value for total ammonia during experimental period was 0.43±0.30mgL^{1}. Dissolved oxygen recorded during 12 weeks experimental period fortnightly was non-significant and the mean value observed as 6.79±0.29 mgL^{-1}. The mean value recorded for electrical conductivity was 2.92±0.18 $mScm^{-1}$. The average values for calcium and magnesium contents were recorded 15.33 ± 0.36 and 40.18 ± 0.33 mgL^{-1}.

The temperature observed among all fortnights was non-significant with the mean value of 30.01 ±0.33ºC. The pH was recorded as non-significant among all fortnights with the mean value of 7.07 ±0.33. Total hardness recorded during 6 fortnights was non-significant and the mean value was 199.67 ±1.33 mgL^{-1}. Total ammonia and dissolved oxygen were remained non-significant with the mean values of 0.51 ± 0.18 and 6.51 ± 0.24 mgL^{-1}. Electrical conductivity was observed 2.66 ± 0.08 $mScm^{-1}$. The calcium and magnesium contents for this treatment were recorded 15.26 ± 0.41 and 40.26 ± 0.40 mgL^{-1}, respectively.

The temperature and pH values were observed as 29.98 ± 0.22ºC and 7.01 ± 0.12, respectively. The total hardness recorded during 6 fortnights was non-significant and the mean value was 199.95 ±1.29. Total ammonia remained under tolerance level of fish with the mean value recorded as 0.76 ± 0.25 mgL^{-1}. Dissolved oxygen was recorded as 6.70 ± 0.38mgL^{-1}. Electrical conductivity remained within the range of 2.50 ± 0.36 – 2.72 ± 0.49$mScm^{-1}$. The calcium and magnesium contents remained 14.83 ± 0.57 and 40.31 ± 0.52 mgL^{-1}.

Like all treated groups, the physico-chemical parameters remained statistically non-significant throughout the experimental trial. The average value of temperature was observed as 29.90 ± 0.23ºC. The pH value for treatment among all fortnights was non-significant and the mean value recorded was 7.03±0.11. Total hardness was 199.61 ± 0.97mgL^{-1}, Total ammonia and Dissolved oxygen values were recorded as 0.46 ± 0.13 and 6.28 ± 0.14mgL^{-1}. The average value of electrical conductivity was recorded 2.54 ± 0.12 $mScm^{-1}$. The calcium and magnesium contents were observed as 15.10 ± 0.37 and 40.26 ± 0.36 mgL^{-1}, respectively.

The overall physico-chemical parameters were observed statistically non-significant and their values are presented as Table 6.

DISCUSSION

Supplementation of diet with different concentrations of *B. subtilis* in the present study resulted to feed utilization, better growth, hematological improvement and body composition of fish (Table 3, Table 4, and Table 5). In the recent study Opiyo *et al.* (2019) reported the effect of *S. cerevisiae* and *B. subtilis* on Nile tilapia (*Oreochromis niloticus*) growth, feed utilization. Another study has been resulted probiotics may enhance the feed digestion, growth, and improve the gut enzyme, and microbiota (Merrifield *et al.,* 2010; Welker and Lim, 2011). A study in India determined the effect of *L. rhamnosus* and *B. subtilis* on *L. rohita* weight gain, feed consumption, biochemical analysis, gut microflora and antibacterial activity of probiotics (Munirasu *et al.,* 2017). The present study results reviewed with Hamdan *et al.* (2016) the fish fed with probiotics *Lactobacillus* sp strain enhance the body weight, total protein, crude lipid profile in Nile tilapia and Hisar *et al.* (2015) suggested *B. subtilis* and *L. plantarum* as good feed additive to improve the growth, body composition, hematological parameters and immune response of *Oncorhynchus mykiss*.

The current study results also compared with findings of Giri *et al.* (2014) reported that *L. rohita* fed with probiotics *B. subtilis*, *P. aeruginosa*, *B. subtilis*, *L. plantarum* enhance the growth performance. Lara-Flores *et al.* (2003) used *L. acidophilus* and *S. cerevisiae* in fish feed and resulted the significant growth rate in *O. niloticus*. A study revealed that commercial feed contains *B. subtilis* increased the growth rate and lower FCR in *O. niloticus* (El-Haroun*et al.,* 2006). Hassaan *et al.* (2014) examined significant final weight again and better FCR of *O. niloticus* fed with different concentration of yeast. Study in *Poecilia reticulate* and *Xiphophorus helleri* fed with *B. subtilis* found the improvement in growth and an enzyme amylases and proteases activity (Ghosh *et al.,* 2008). Fingerlings of Indian major carp (*L. rohita*) and grass carp (*C. idella*) fed with *Lactobacillus* and *Bacillus* spp, showed higher marketed growth (Kumar *et al.,* 2006; Wang, 2011). The results of

present study corroborated the findings of previous studies which reported an augmented growth rate of the fish species; *O. mykiss*, *O. niloticus*, and *C. carpio* fed with probiotics *B. subtilis* and *S. cerevisiae* (Yanbo and Zirong, 2006; Tawwab *et al.*, 2008; Hassaan *et al.,* 2014; Adel *et al.*, 2017). Munir *et al.* (2016) reported the significant growth rate, feed utilization and survival rate in snakehead (*C. striata*) fed with dietary probiotics (*S. cerevisiae* and *L. acidophilus*). Comparable results were observed in earlier studies (Vendrell *et al.,* 2008; Son *et al.,* 2009; Merrifield *et al.,* 2010) which evaluated the impact of probiotics *L. plantarum* and *B. subtilis* on intestinal microbiota and growth in different fishes. Another study evaluated that the additions of probiotic augment the size, growth and survival rate of salt water fish larvae (Blain *et al.,* 1998). Juvenile *Dentex* were fed with two different probiotics but reported non-significant comparison between the treatment group and control group (Hidalgo *et al.,* 2006). The results of the present study have close similarity with previous examination by Abdel-Tawwab and Ahmad (2009) where use of live *Spirulina* showed significant FCR than control treatment. The lower FCR were recorded for *L. rohita* fed with probiotics containing diets (Mohapatra *et al.,* 2012).

Percentage of crude protein, crud fats, ash, carbohydrates in T1, T2, and T3 fed with *B. subtilis* were statistically significant than control group. The same findings were studied in *C. carpio* and rainbow trout fed with different kind of probiotics (Farzanfar *et al.,* 2007). Another study reported non-significant effect of probiotic on protein, moisture and ash content of Nile tilapia. In addition, the significant improvement of carcass fat has been reported (Lara-Flores *et al.,*2003; Ghosh *et al.,*2008). Azarin *et al.* (2015) examined the effect of *B. licheniformis*, *B. subtilis*, and ferroin on *Rutilus frisii kutum* and concluded higher protein level, on the other hand lipid, moisture and ash level were reported non-significant. *B. subtilis* and *B. circulans* were used in *L. rohita* feed to find the effect on protein efficiency ratio (PER), growth, FCR and reported significantly FCR, growth and PER (Bairagi *et al.,* 2004). The present study results have close resemblance with pervious study by Wang and Xu, (2006) tested *Bacillus* spp and photosynthetic bacteria in common carp feed and reported highly significant growth performance and FCR, then control groups.

In the present study, hematological (TLC, Total RBC, Hemoglobin, Platelets, Neutrophil, Lymphocytes, Monocytes, Eosinophils, Total protein, Albumin, Globulin) parameters in *L. rohita* fed with different levels (T1, T2, T3 and control) of probiotics are found highly significant, which has the similarity with results evaluated by Azarin *et al.* (2015). Another study revealed that *O. mykiss* fed with *L. acidophilus* enhanced Hb and RBC values as compared to the control group (Faramarzi *et al.,* 2011). Use of *Bacillus* sp. in fish diet induced the development of immunity system against different the disease in fish (Siwicki *et al.,* 1994; Brunt *et al.,* 2007; Rengpipat *et al.,* 2008). Another study reported the application of *L. lactis* and *L. rhamnosus* in red seabream and concluded the significant results (Dawood *et al.,* 2017), in Nile tilapia fed diet *Bacillus* spp, were used as additive (Garcia-marengoni *et al.,* 2015; Feliatra *et al.,* 2018) and *L. rhamnosus have been tested* (Kiron and Watanabe, 2010) *in fed diet of* rainbow trout, all previous studies found higher level of Hct. Munir *et al.* (2018) reported the significant effects of probiotic based diets on WBC levels and immune defense. *L. sporogenes* have been used in *C. batrachus* feed and caused higher levels of WBC (Dahiya *et al.,* 2012). *L. rohta* under feed trial supplemented with *B. Pamillus* yielded the higher level of Hb (Rajikkannu *et al.,* 2015). In another study *Rutilis frisii* kutum has been fed with feed having *B. subtilis* and *B. Licheniforms and concluded significant results of* Hemoglobin (Azarin *et al.,* 2015). All the physico-chemical parameters of water described in results showed that all mean values were non-significant among all treatments which reveal that fish present in all treatments have same water condition due to which the comparison of growth and health of fish were recorded in best way.

***Conclusion*:** The present study delegated that the probiotics in fish feed lessen the FCR, increases growth parameters and immunologic level. The study concluded that *B. subtilis* as supplement in fish diet may enhance the growth rate with favorable economic condition.

Authors Contributions statement: 1st & 2nd authors contributed their 100%, 3rd, 4th& 5th contributed 30% and last 2-authors contributed their 15%

Conflict of interest: Not Any

***Acknowledgement*:** Authors are thankful to the Department of Fisheries & Aquaculture, UVAS, Lahore for provision of facilities to carry out the research work for this manuscript.

REFERENCE

Tawwab, A., M. A. Rahman and N. E. Ismael. 2008. Evaluation of commercial live bakers' yeast, *Saccharomyces cerevisiae* as a growth and immunity promoter for Fry Nile tilapia, *Oreochromis niloticus* (L.) challenged in situ with *Aeromonas hydrophila*. Aquaculture. 280:185-189.

Tawwab, A., M. and M. H. Ahmad. 2009. Live Spirulina (*Arthrospira platensis*) as a growth and immunity promoter for Nile tilapia, *Oreochromis niloticus* (L.), challenged with pathogenic *Aeromonas hydrophila*. Aquaculture Research. 40:1037-1046.

Adel, M., Lazado, C. C. Safari, R. Yeganeh and M. J. Zorriehzahra. 2017. Aqualase, a yeast-based in-feed probiotic, modulates intestinal microbiota, immunity and

growth of rainbow trout *Oncorhynchus mykiss*. Aquaculture Research. 48:1815-1826.

AOAC., 2006. Official Method of Analysis. 18th Edt. Washington, D.C: Association of Official Analytical Chemist.

Azarin, H., M. S. Aramli, M. R. Imanpour and M. Rajabpour. 2015. Effect of a probiotic containing *Bacillus licheniformis* and *Bacillus subtilis* and ferroin solution on growth performance, body composition and hematological parameters in *Kutum* (*Rutilus frisii kutum*) fry. Probiotics Antimicrobiology. 7:31-37.

Bairagi, A., S. Ghosh, K. Sen and A.K. Ray. 2004. Evaluation of the nutritive value of *Leucaena leucocephala* leaf meal, inoculated with fish intestinal bacteria *Bacillus subtilis* and *Bacillus circulans* in formulated diets for rohu, *L. rohita* (Hamilton) fingerlings. Aquaculture Research. 35:436-446.

Blain, K., S. Tucker, J. W. Neidig, C. L. Vermeer, G. K. Cooper, V. R. Jarrell and D. G. Sennett. 1998. Bacterial management strategies for stock enhancement of warm water marine fish: A case study with common snook (*Centropomus undecimalis*). Bulletin of Marine Science. 62: 573-588.

Brunt, J. A., N. Fyzul and B. Austin. 2007. The development of probiotics for the control of multiple bacterial diseases of rainbow trout, *Oncorhynchus mykiss* (*Walbaum*). Journal of Fish Disease. 30: 573-579.

Cazorla, F., M. Romero, D. P. Garcia, A. Lugtenberg, B. J. J. Vicente, A. D. and G. Bloemberg, 2007. Isolation and characterization of antagonistic *Bacillus subtilis* strains from the avocado rhizoplane displaying biocontrol activity. Journal of Applied Microbiology. 103:1950-1959.

Chauhan, A. and R. Singh. 2019. Probiotics in aquaculture: a promising emerging alternative approach. Symbiosis. 77: 99-113.

Dahiya, T., R.C. Sihag and S. Gahlawat. 2012. Effect of probiotics on the haematological parameters of Indian magur {*Clarius batrachus (L)*}. Journal of Fisheries and Aquatic Science. 7: 279–290.

Dawood, M.A.O., S. Koshio, M. Ishikawa, S. Yokoyama and M. F. E. Basuini. 2017. Effects of dietary supplementation of *Lacto bacillus rhamnosus* or/and *Lactococcus lactis* on the growth, gut microbiota and immune responses of red sea bream, *Pagrus major*. Fish and Shellfish Immunology. 49:275-285.

Duncan, P.L. and P.H. Klesius. 1996. Dietary immune a stimulant enhances nonspecific immune responses in channel catfish but not resistance to *Edward siellaictaluri*. Journal of Aquaculture. 8: 241-248

Haroun, E. R., A.S. Goda and M. A. K. Chowdhury. 2006. Effect of dietary probiotic Biogen supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (L.). Aquaculture Research. 37:1473-1480.

FAOSTAT, F. Statistics, Food and Agriculture Organization of the United Nations, Rome. 2020.

Faramarzi, M., S. Kiaalvandi, M. Lashkarbolooki and F. Iranshahi. 2011. The investigation of Lactobacillus acidophilus as probiotics on growth performance and disease resistance of rainbow trout (*Oncorhychus mykiss*). American *Eurasian* Journal of Agriculture and Environmental Sciences. 6: 32-38.

Farzanfar, A., A.G. Lashto, M. Alizadeh, M. Bayati and R. Ghorban. 2007. Study on growth performance of Rainbow trout, *Oncorhynchus mykiss*, larvae with different concentration of probiotic in diet. Proceedings of Aquaculture, San Antonio, Texas, USA.

Feliatra, F., L. Elizal, I. Lukistyowati, D. Melina and M. Ramadhani. 2018. Effectiveness of Immersion with Probiotic in Improving the Health of Nile Tilapia (*Oreochromis niloticus*). Asian Journal of Animal and Veterinary Advances. 13:43-51.

Foysal, M. J. and A. K. Lisa. 2018. Isolation and characterization of *Bacillus sp.* strain BC01 from soil displaying potent antagonistic activity against plant and fish pathogenic fungi and bacteria. Journal of Genetic Engineering and Biotechnology. 16: 387-392.

Fuller, R. 1992. History and development of probiotics. In Probiotics Springer, Dordrecht.1-8.

Garcia, M. N, M.C. Moura, N. Tavares and E. D. Oliveira. 2015. Short Communication Use of probiotics *Bacillus cereus* var. toyoi and *Bacillus subtilis* C-3102 in the diet of juvenile Nile tilapia cultured in cages. Latin American Journal of Aquatic Research. 43: 601-606.

Ghosh, K., S.K. Sen and A.K. Ray. 2002. Characterization of *Bacillus* isolated from the gut of rohu, *Labeo rohita*, fingerlings and its significance in digestion. Journal of Applied Aquaculture. 12: 33-42.

Ghosh, S., A. Sinha and C. Sahu. 2008. Dietary probiotic supplementation in growth and health of live-bearing ornamental fishes. Aquaculture Nutrition. 14:289-299.

Ghosh, S. Sinha, A. and C. Sahu. 2008. Dietary probiotic supplementation in growth and health of live-bearing ornamental fishes. Aquaculture Nutrition. 14:289-299.

Giri, S.S., V. Sukumaran, S.S. Sen and P.K. Jena. 2014. Effects of dietary supplementation of potential probiotic *Bacillus subtilis* VSG1 singularly or in combination with *Lactobacillus plantarum* VSG3 or/and *Pseudomonas aeruginosa* VSG2 on the growth, immunity and disease resistance of *Labeo rohita*. Aquaculture Nutrition. 20:163-171.

Gram, H.C. 1884. "Uber die isolierte Farbung der Schizomyceten in Schnitt and Trockenpräparaten". Fortschritte der Medicine (in German). 2:185-189.

Green, D.H., P.R. Wakeley, A. Page, A. Barnes, L. Baccigalpi, E. Ricca and S.M. Cutting. 1999.

Characterization of two *Bacilus* probiotics. Applied Environmental Microbiology. 65: 4288-4291.

Hamdan, A. M., A.F.E Sayed and M. M. Mahmoud. 2016. Effects of a novel marine probiotic, *Lactobacillus plantarum* AH 78, on growth performance and immune response of Nile tilapia (*Oreochromisniloticus*). Journal of Applied Microbiology. 120:1061-1073.

Hassaan, M. S., M.A. Soltan and M. M. R. Ghonemy. 2014. Effect of symbiotic between *Bacillus licheniformis* and yeast extract on growth, hematological and biochemical indices of the Nile tilapia (*Oreochromis niloticus*). Egyptian Journal of Aquaculture Research. 40:199-208.

Hidalgo, M. C., A. Skalli, E. Abellan, M. Arizcun and G. Cardenete. 2006. Dietary intake of probiotics and maslinic acid in juvenile dentex (*Dentex dentex L.*): effects on growth performance, survival and liver proteolytic activities. Aquaculture Nutrition. 12:256-266.

Hisar, O., S. Yilmaz and M. Yigit. 2015. Effects of different probiotic bacteria on growth, body composition, immune response and hematological parameters of rainbow trout (*Oncorhynchus mykiss*) under sublethal water temperature. Journal of Marine Science. Technology. 4:21-28.

Hopkins, K.D. 1992. Reporting fish growth: A review of the Basics. Journal of World Aquaculture Society. 23: 173-179.

Hoseinifar, S.H., Y.Z. Sun, A. Wang and Z. Zhou. 2018. Probiotics as Means of Diseases Control in Aquaculture, a Review of Current Knowledge and Future Perspectives. Frontiers of Microbiology. 9:2429.

Irianto, A. and B. Austin. 2002. Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchusmykiss* (Walbaum). Journal of Fish Diseases. 25:333-342.

Jayaprakash, S., K. Parvathi and C. Kalamani. 2019. Influences of Isolated Gut Probiotic *Bacillus subtilis* on Growth Performance of Freshwater fish *Labeorohita*. International Journal of Life Sciences Research. 6:342-348.

Kalarani, V., V. Sumathi and D.C. Reddy. 2015. Effect of dietary supplementation of *Bacillus subtilis* and *Terribacillus saccharophillus* on the innate immune responses of a tropical freshwater fish, *L. rohita*. Journal of Clinical Cell Immunology. 6:82.

Kavitha, M., M. Raja and P. Perumal. 2018. Evaluation of probiotic potential of *Bacillus* spp. isolated from the digestive tract of freshwater fish *Labeo calbasu* (Hamilton, 1822). Aquaculture Reports. 11:59-69

Kiron, A.P.V. and S.S.T Watanabe. 2010. Probiotic bacteria *Lactobacillus rhamnosus* influences the blood profile in rainbow trout *Oncorhynchus mykiss* (Walbaum). Fish Physiology Biochemistry. 36:969-977.

Kuebutornye, F.K.A., E.D.L Abarike, Y. Hlordzi, V. Sakyi, M.E. Afriyie, G. Wang, Z. Y. Li and C.X. Xie. 2020. Mechanisms and the role of probiotic *Bacillus* in mitigating fish pathogens in aquaculture. Fish Physiology and Biochemistry. 46: 819-841.

Kumar, R., S.C. Mukherjee, K.P. Prasad and A.K. Pal. 2006. Evaluation of *Bacillus subtilis* as a probiotic to Indian major carp *L. rohita* (Ham.). Aquaculture Research. 37: 1215-1221.

Lara, F.M., N.M.A. Olvera, B.E.M. Guzman and W.L. Madrid. 2003. Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). Aquaculture. 216:193-201.

Mac, F.J.F., W. Wilkins and Baltimore. 1980. Biochemical Tests for Identification of Medical Bacteria.

Matiullah, F. Rasool, E.M. Bhatti, N. Khan and S. Parveen. 2016. Use of black pepper seed as growth enhancer in *L. rohita*. International Journal of Bioscience. 8:1-9.

Merrifield, D. L., G. Bradley, R.T.M. Baker and S. J. Davies. 2010. Probiotic applications for rainbow trout (*Oncorhynchus mykiss Walbaum*) II. Effects on growth performance, feed utilization, intestinal microbiota and related health criteria post antibiotic treatment. Aquaculture Nutrition. 16:496-503.

Merrifield, D.L., A. Dimitroglou, A. Foey, S.J. Davies, R.T. Baker, J. Bogwald and E. Ringo. 2010. The current status and future focus of probiotic and prebiotic applications for salmonids. Aquaculture. 302:1-18.

Michael, S.E., E.D. Abarike, E.D. and J. J. Cai. 2019. A Review on the Probiotic Effects on Haematological Parameters in Fish. Journal of Fisheries Science and commerce. 13:25-31.

Mohapatra, S., T. Chakraborty, K. Ashisa, P. Kurchetti, P. Prasad, N. Kedar and K. N. Mohanta. 2014. Beneficial Effects of Dietary Probiotics Mixture on Hemato-Immunology and Cell Apoptosis of *L. rohita* Fingerlings Reared at Higher Water Temperatures. *PloS one.* 9:100-929

Mohapatra, S., T. Chakraborty, V. Kumar, G. DeBoeck and K. Mohanta. 2013. Aquaculture and stress management: a review of probiotic intervention. J. Anim. Physiol. Nut. 97:405-430.

Mohapatra, S., T. Chakraborty, A.K. Prusty, P. Das, K. Paniprasad and K.N. Mohanta. 2012. Use of different microbial probiotics in the diet of rohu, *L. rohita* fingerlings: effects on growth, nutrient digestibility and retention, digestive enzyme activities and intestinal microflora. Aquaculture Nutrition. 18:1-11.

Munir, M.B., R. Hashim, M.S.A. Manaf and S.A.M. Nor. 2016. Dietary prebiotics and probiotics influence the growth performance, feed utilization, and body indices of snakehead (*Channa striata*) fingerlings. Tropical Life Science Research. 27:111.

Munir, M.B., R. Hashim, S.A.M. Nor and T.L Marsh. 2018. Effect of dietary probiotics and probiotics on snakehead

(*Channa striata*) health: Hematology and disease resistance parameters against *Aeromonas hydrophila*. Fish Shellfish Immunology. 75:99-108.

Munirasu, S., V. Ramasubramanian and P. Arunkumar. 2017. Effect of Probiotics diet on growth and biochemical performance of freshwater fish *L. rohita* fingerlings. Journal of Entomology and Zoological Studies. 5:1374-1379.

Nandi, A., G. Banerjee, K. Dan, K. Ghosh and K. Ray. 2017. Probiotic efficiency of *Bacillus* sp. in *L. rohita* challenged by *Aeromonas hydrophila*: assessment of stress profile, haemato-biochemical parameters and immune responses. Aquaculture Research. 48: 4334-4345.

Nayak, S.K., P. Swain and S.C Mukherjee. 2007. Effect of dietary supplementation of probiotic and vitamin on the immune response of Indian major carps, *L. rohita*. Fish Shellfish Immunology. 23:892-896.

Olivie, G., R. Lallier and S. Lariviere. 1981. At oxigenic profile of *Aeromonas hydrophila* and *Aeromonas sobria* isolated from fish. Canadian Journal of Microbiology. 27:330-333.

Opiyo, M.A., J. Jumbe, C.C. Ngugi and H.C. Karisa. 2019. Different levels of probiotics affect growth, survival and body composition of Nile tilapia (*Oreochromis niloticus*) cultured in low input ponds. Scientific African.4:103.

Panigarhi, A. and Azad. 2007. Microbial intervention for better fish health in aquaculture: The Indian scenario. Fish Physiology and Biochemistry. 33:429-440.

Rajesh, L., D. Maharajh, J. Gorgens and N. Gardiner. 2008. Functionality of a *Bacillus cereus* biological agent in response to physiological variables encountered in aquaculture. Applied Microbiology and Biotechnology. 79:111-118.

Rajikkannu, M., N. Natarajan, P. Santhanam, B. Deivasigamani and S. Janani. 2015. Effect of probiotics on the hematological parameters of Indian major carp (*L. rohita*). International Journal of Fisheries Aquatic Studied. 2:105-109.

Ramesh, D. and S. Souissi. 2018. Effects of potential probiotic *Bacillus subtilis* KADR1 and its subcellular components on immune responses and disease resistance in *L. rohita*. Aquaculture Research. 49:367-377

Ramesh, D. and S. Souissi. 2018. Effects of potential probiotic *Bacillus subtilis* KADR1 and its subcellular components on immune responses and disease resistance in *L. rohita*. Aqua. Res. 49:367-377.

Rengpipat, S., W. Phianphak, S. Piyatiratitvoraku and P. Menasveta. 1998. Effects of probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. Aquaculture. 167:301-313

Rengpipat, S.T., Rueangruklikhit and S. Piyatiratitivorakul. 2008. Evaluations of lactic acid bacteria as probiotics for juvenile seabass *Lates calcarifer*. Aquaculture Research.39: 134-143.

Robertson, P.A.W., C. Dowd, C. Burrell, P. Williams and B. Austin. 2000. Use of Carnobacterium sp. as a probiotic for Atlantic salmon (Salmo salar L.) and rainbow trout (Oncorhynchus mykiss, Walbaum). Aquaculture. 185:235-243.

Roos, N., M.A. Wahab, C. Chamnan and S.H. Thilsted. 2007. The role of fish in food-based strategies to combat vitamin A and mineral deficiencies in developing countries. Journal of Nutrition. 137:1106-1109.

Saini, V.P., M.L. Ojha, M.C. Gupta, P. Nair, A. Sharma and V. Luhar. 2014. Effect of dietary probiotics on growth performance and disease resistance in *L. rohita* fingerling. International Journal of Fisheries and Aquatic Studies. 1:7-11.

Sakai, M. 1999. Current research status of fish immunostimulants. Aquaculture. 172: 63-92.

Schon, H.E., I. Bogut and I. Strelec. 2006. Heavy metal profile in five fish species included in human diet, domiciled in the end flow of river Neretva (*Croatia*). Archives of Environmental Contamination and Toxicology. 50:545-551.

Siwicki, A. K., D.P. Anderson and G.L. Rumsey. 1994. Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. Veterinary Immunology and Immunopathology. 41:125-139.

Stasiack, A.S. and C.P. Baumann. 1996. Neutrophil activity is a potent indicator of concomitant analysis. Fish and ShellFish Immunology. 6:537.

Steel, R.G.D., J.H. Torrie and D.A. Dinkey. 1996. Principles and procedure of statistics, 2nd Ed, McGraw Hill Book co Singapur.

Vendrell, D., J.L. Balcazar, D.I. Blas, I.R. Zarzuela, O. Girones and J.L. Muzquiz. 2008. Protection of rainbow trout (*Oncorhynchus mykiss*) from *lactococcosis* by probiotic bacteria. Comparative Immunology, Microbiology and Infectious Diseases. 31:337-345.

Wang, Y. 2011. Use of probiotics *Bacillus coagulans*, *Rhodopseudomonas palustris* and *Lactobacillus acidophilus* as growth promoters in grass carp (*Ctenopharyngodon idella*) fingerlings. Aquaculture Nutrition. 17:372-378.

Wang, Y. and Z.R. Xu. 2006. Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. Animal Feed Science and Technology. 127: 283-292

Wang, Y.B., Z.R. Xu and M.S. Xia. 2005. The effectiveness of commercial probiotics in northern white shrimp *Penaeus vannamei* ponds. Fisheries Science. 71:1036-1044.

Wang,Y.B., J.R. Li and J. Lin. 2008. Probiotics in aquaculture: challenges and outlook. Aquaculture. 281:1-4.

Welker T.L. and C. Lim. 2011. Use of probiotics in diets of tilapia. Aquaculture Research and Development. 1:1-8

Wulff, E.G., C.M. Mguni, G.K. Mansfeld, J. Fels, M. Lubeck and J. Hockenhull. 2002. Biochemical and molecular characterization of *Bacillus amyloliquefaciens*, *B. subtilis* and *B. pumilus* isolates with distinct antagonistic potential against *Xanthomonas campestris pv*. campestris. Plant Pathology. 51:574-584.

Yanbo, W. and X. Zirong. 2006. Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. Animal Feed Science and Technology. 127: 283-292.