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INFLUENCES OF ISOLATED GUT PROBIOTIC *BACILLUS SUBTILIS* ON BIOCHEMICAL PARAMETERS OF THE INDIAN MAJOR CARP *LABEO ROHITA* (HAMILTON, 1822)

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ABSTRACT

To find out the effect of isolated single probiotics *Bacillus subtilis* on the biochemical parameter of freshwater fish *Labeo rohita*. The advisable impact of isolated probiotic *Bacillus subtilis* (SUB 3845847 SeqJP2 MH128358) on the biochemical parameters such as blood glucose, total protein, cholesterol, albumin and globulin were analyzed in the freshwater fish *Labeo rohita* for the experimental period of 60 days. The two tubs such as control tub (fed without probiotics) and experimental tub (feed fed with *Bacillus subtilis* (SUB 3845847 SeqJP2 MH128358)) was maintained and 30 fingerling freshwater fish *Labeo rohita* were introduced into each tub and continuous aeration was given, Each tub treatment had triplicates. The feed used to be given twice a day morning 6 am and evening 6 pm commonly at the rate of 3% physique weight and water used to exchange every day with the water pump. At the end of the day, blood samples were gathered at the intervals 15th, 30th, 45th and 60th days. Results indicate that the biochemical parameters was improved in the experiment tub than the control tub. The results suggest that isolated probiotic *Bacillus subtilis* have improved the value of the biochemical parameter of the freshwater fish *Labeo rohita*.

KEYWORDS: Gut probiotic, Biochemical parameters, Fingerlings *Labeo rohita* and Formulated diet.

INTRODUCTION

Probiotics are the beneficial microbes promoting growth performance and protecting the host against pathogens. Isolated probiotic *Bacillus subtilis* with formulated diet improved digestive activity and stimulate the growth of freshwater fish *Labeo rohita* (Jayaprakash *et al*., 2019). The probiotic bacteria as live microorganisms which when managed in acceptable quantities discuss a health benefit (FAO/WHO, 2001).The species which are to be used more include the *Bacillus subtilis, Bacillus coagulants* and *Bacillus cereus.* Which when they are used as the probiotics in form of the feeding enhancement, they will lead to the encouragement of the immune system and have the antimicrobial activity and prevention (Cutting, 2010 and Farzanfar *et al*., 2009). The blood parameters of fish offer correct indications of any changes occurring within the organism as a result of injuries to organs or tissue associated with communicable disease, similar to those of warm-blooded animals (Aderfemo *et al*., 2007). It was reported that the hematologic and organic chemistry parameters of fish may be wont to judge the health condition of the organism. The microorganisms used as probiotics, including *Lactobacillus*, *Bacillus* and *Yeasts*, have been reported in penaeids and fish (Pooramini *et al*., 2009 and Capkin *et al*., 2009). Probiotics play important roles as immunostimulants and antimicrobial agents. The probiotics area unit live microorganism or genteel product feed supplements that beneficially have an effect on the host by manufacturing repressive compounds, competing for chemicals and adhesion sites, modulating and stimulating immune function, and improving microbial balance (Schaperclaus *et al*., 1992). Among this, *Labeo rohita* contributes a major portion to the freshwater fish production in South India. The aim of the present study was to evaluate the effects of probiotics the bacteria *Bacillus subtilis* biochemical performance of freshwater fish *Labeo rohita* for with the following objectives.

MATERIAL METHOD

Sample Collection and Acclimatization

Healthy, freshwater fish *Labeo rohita* (5.00 gm weight and 4.5 cm length) were purchased from Government fish farm, Bhavanisagar, Erode District, Tamil Nadu and acclimatized in laboratory conditions in a plastic tub (25^{o}

C/77^{o} F, pH 7.0) with continuous aeration for three weeks prior to the commencement of the experiment. Stocked fish were fed with supplementary diet ad libitum (AOAC, 1990).

Isolation of culturable intestinal microbiota
The freshwater fish *Labeo rohita* were washed with sterile water and dissected to remove the fish intestine by the sterilization condition. The fish intestine was homogenized in the same sterile distilled water and centrifugation, next centrifugation supernatant was taken and serially diluted in sterile distilled water in the test tubes. The homogenized intestine sample was serially diluted in normal saline solution (NSS) and aseptically plated by the spread plate technique on a nutrient agar plate and incubated for 24 hours room temperature to determine to the plate count (Whiteman *et al*., 2009). Selective colonies were characterized and known following Bergey's Manual of Systematic Bacteriology (Whiteman *et al*., 2009) for this or her colony and cell morphology, gram staining, organic chemistry and physiological tests (Mandal *et al*., 2008 and Ghosh *et al*., 2003). Finally, the 16S rRNA sequence was deposited in the NCBI GenBank nucleotide sequence database under accession number *Bacillus subtilis* (SUB 3845847 SeqJP2 MH128358).

Feed preparation and proximate composition
To prepare the diet, the following ingredients such as (Rice bran 40%, Groundnut oil cake 20%, Dry fish meal 15%, Soya meal 15%, Maize 9%, Vitamins & Minerals mix 1%) were purchased from local Erode market, Tamil Nadu, India. All the ingredients were mixed and powdered by a machine and were made into dough with the help of distilled water (Sivakumar et al., 2014). Feed ingredients and experimental pellet diet were analyzed for the proximate composition according to (AOAC, 1990) procedure as follows; Carbohydrate, Protein, Lipid, Moisture, and Ash content was a determination for (AOAC, 1990) protocols (Table 1).

Table 1: Proximate Composition (% dry matter basis) analysis of experimental diets.

Ingredients	Control diet (%)	Experiment diet (%)
Rice bran	40	40
Groundnut	20	20
Fish meal	15	15
Soya Meal	15	15
Maize	9	8
Vitamin & Mineral mix	1	1
Bacillus subtilis	-	1
Proximate Composition (%)		
Dry matter	99.90	99.80
Carbohydrate	20.72	21.23
Protein	35.65	35.53
Lipid	9.16	9.14
Moister	9.34	9.40
Ash	13.14	14.10
Digestible energy (k.cal/kg)	3294.27	3258.45

(-) The absence of *Bacillus subtilis*

Experimental design
Experimental fish were divided into two groups such as the control group and the experimental group. Fish in the control tub were fed only with formulated feed (without any probiotic), fish in the experimental tub was fed with formulated feed mixed with isolated probiotic bacteria *Bacillus subtilis*. Each treatment had triplicates. The fish were fed with a formulated diet was given twice a day morning 6 am and evening 6 pm regularly at the rate 3% body weight and water changed every day with the water pump (Dada *et al*., 2002). The experiment was conducted on 15th, 30th, 45th, and 60th days (AOAC, 1990).

Control - Formulated diet only

Experiment - Formulated diet + Probiotic bacteria (*Bacillus subtilis)*

Biochemical parameters
Two ml of blood samples were collected using sterilized syringes from the caudal vein. The blood was collected in CLOT activator tubes and transported to the laboratory for biochemical parameters were analyzed at the end intervals of 15th, 30th, 45th and 60th days. The blood sample was collected and the blood samples were centrifuged for 5min and serum was separated, the supernatant plasma was used for the determinations of the plasma biochemical parameters. The biochemical parameters were analyzed using Cobas Integra 400 plus biochemistry analyzer was used for diagnostic clinical chemistry testing this method (Schlam *et al*., 1975 and Mitruka *et al*., 1977). The blood glucose concentration was estimated by glucose oxidase-peroxidase (GOD-POD) method (Trinder, 1969). The total cholesterol was estimated by cholesterol oxidase peroxidase (GOD-POD) method (Allain *et al*., 1974). The blood samples were subjected to determine plasma total protein and albumin

(Tietz, 1990). Although globulin concentrations were determined by deducting the concentration of total protein from albumin concentration.

Statistical Analysis

Means ± SD with the different superscripts within a column is significantly (P<0.05) One-way analysis of variance ANOVA by using SPSS 16.0 software.

RESULTS

The total serum biochemical parameter of *Labeo rohita* fed with formulated diet (without probiotic) and fed with formulated diet (isolated probiotic *Bacillus subtilis)* was shown in Tables 2 and Figure 1. The blood samples were analyzed on 15th, 30th, 45th and 60th day throughout the experimental period. The blood glucose in the control group is found to be a minimum increased the value of 13.52 ± 1.0, 14.10 ± 0.80, 26.20 ± 1.72 and 27.25 ± 1.52 (gm/dl) respectively. The blood glucose in the experiment group is found to be a maximum increased the value of 14.25 ± 1.15, 18.07 ± 1.75, 28.30 ± 0.57 and 35.20 ± 1.0 (gm/dl) respectively. The treatment group was significantly (P<0.05) maximum increased in the blood glucose in fish fed with a formulated diet with isolated probiotic *Bacillus subtilis* than the control group.

Table 2: Changes in the blood glucose of freshwater fish *Labeo rohita* fed with a probiotic diet at the different exposure period.

No. of Days	Blood Glucose (gm/dl)	
	Control	Experiment
15	13.52±1.0	14.25±1.15
30	14.10±0.80	18.07±1.75
45	26.20±1.72	28.30±0.57
60	27.25±1.52	35.20±1.0

Values are in mean ±SD

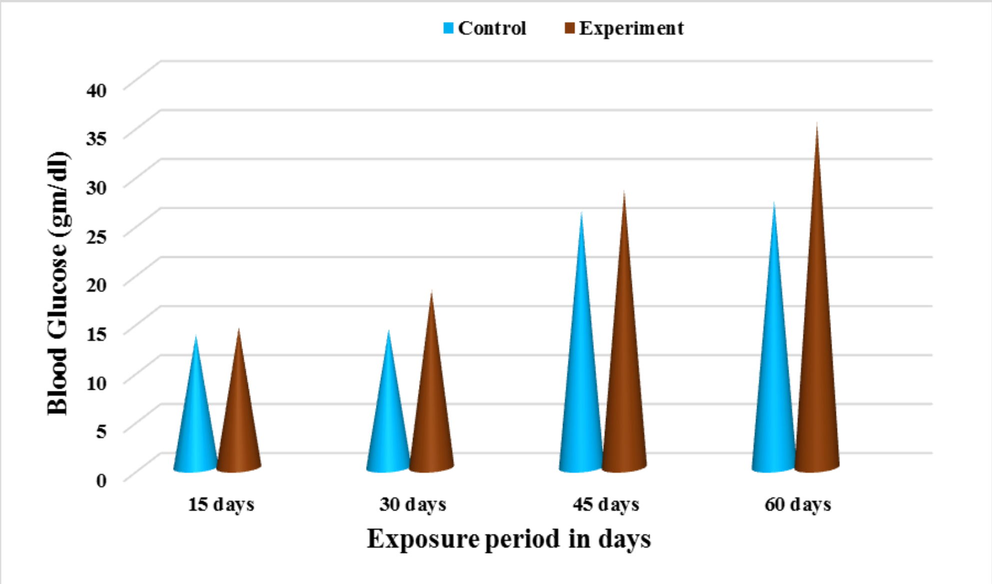

Figure 1: Changes in the blood glucose of freshwater fish *Labeo rohita* fed with a probiotic diet at the different exposure period.

The total protein content in the control fish was found to be as 4.00 ± 0.49, 4.25 ± 0.55, 4.30 ± 1.15 and 5.15 ± 1.15 (gm/dl) of the experimental period respectively. Whereas, the total protein content was recorded as 4.20 ± 0.57, 4.30 ± 0.50, 5.60 ± 1.15 and 6.11 ± 0.57 (gm/dl) in the fish fed with isolated single gut probiotics *B. subtilis* in the experiment.

Table 3: Changes in the total protein of freshwater fish *Labeo rohita* fed with a probiotic diet at the different exposure period.

No. of Days	Total Protein (gm/dl)	
	Control	Experiment
15	4.00±0.49	4.20±0.57
30	4.25±0.55	4.30±0.50
45	4.30±1.15	5.60±1.15
60	5.15±1.15	6.11±0.57

Values are in mean ±SD

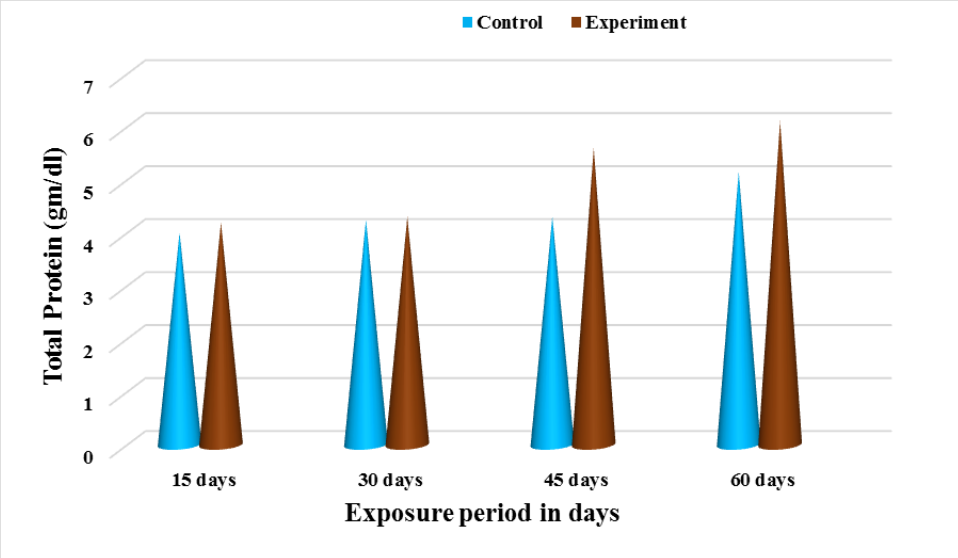

Figure 2: Changes in the total protein of freshwater fish *Labeo rohita* fed with a probiotic diet at the different exposure period.

The total protein content was significantly (P<0.05) maximum increased in the experiment than the control group. The blood cholesterol in the control fish was found to be as 143.50±1.1.5, 144.60±0.57, 145.80±1.73, and 146.90±1.15 (gm/dl) of the experimental period respectively. However, the blood cholesterol was recorded as 148.80±0.57, 149.50±0.82, 150.60±1.18 and 151.80±0.57 (gm/dl) in the fish fed with isolated single gut probiotics *B. subtilis* in the experiment.

Table 4: Changes in the blood cholesterol of freshwater fish *Labeo rohita* fed with a probiotic diet at the different exposure period.

No. of Days	Blood Cholesterol (gm/dl)	
	Control	Experiment
15	143.50±1.1.5	148.80±0.57
30	144.60±0.57	149.50±0.82
45	145.80±1.73	150.60±1.18
60	146.90±1.15	151.80±0.57

Values are in mean ±SD

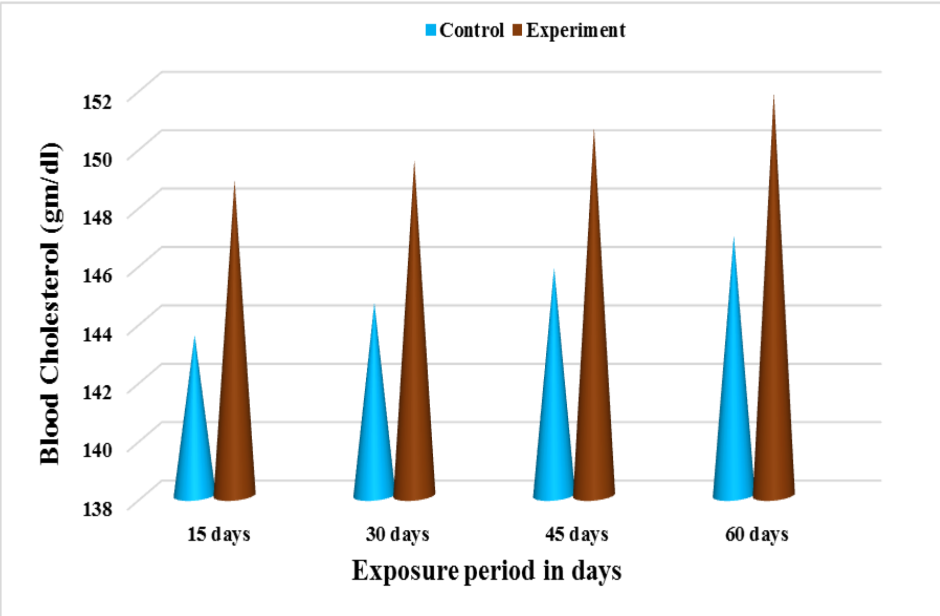

Figure 3: Changes in the blood cholesterol of freshwater fish *Labeo rohita* fed with a probiotic diet at the different exposure period.

The blood cholesterol was significantly (P<0.05) maximum increased in the experiment than the control group. The albumin in the control fish was found to be as 2.8±0.90, 3.2±0.86, 3.3±0.57 and 3.5±0.46 (gm/dl) of the experimental period respectively. However, the albumin was recorded as 3.2±1.27, 3.4±0.80, 3.6±0.46 and 3.9±0.57 (gm/dl) in the fish fed with isolated single gut probiotics *B. subtilis* in the experiment. The albumin was significantly (P<0.05) maximum increased in the experiment than the control group. The globulin in the control fish was found to be as 0.55±0.25, 1.00±0.50, 1.25±0.28 and 1.85±0.86 (gm/dl) of the experimental period respectively. However, the globulin was recorded as 0.60±0.86, 1.30±0.42, 1.80±0.50 and 2.70±0.25 (gm/dl) in the fish fed with isolated single gut probiotics *B. subtilis* in the experiment. The globulin was significantly (P<0.05) maximum increased in the experiment than the control group.

Table 5: Changes in the albumin of freshwater fish *Labeo rohita* fed with a probiotic diet at the different exposure period.

No. of Days	Albumin (gm/dl)	
	Control	Experiment
15	2.8±0.90	3.2±0.86
30	3.2±0.86	3.4±0.80
45	3.3±0.57	3.6±0.46
60	3.5±0.46	3.9±0.57

Values are in mean ±SD

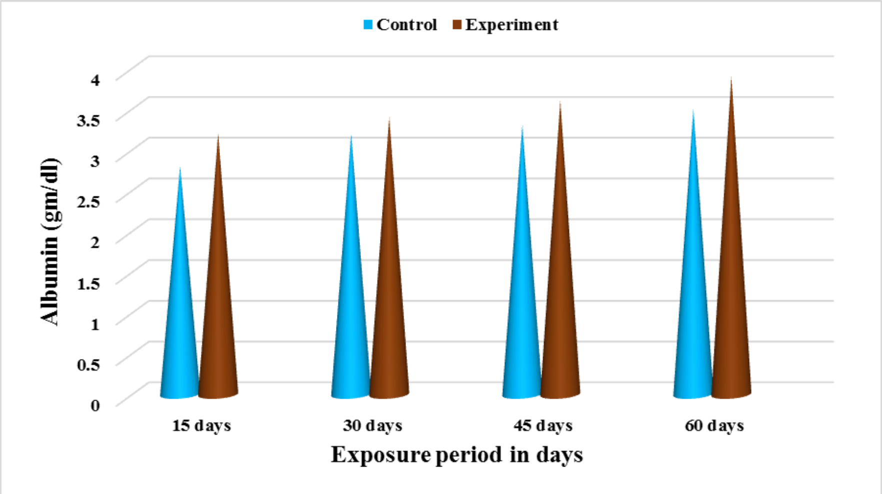

Figure 4: Changes in the albumin of freshwater fish *Labeo rohita* fed with a probiotic diet at the different exposure period.

Table 6: Changes in the globulin of freshwater fish *Labeo rohita* fed with a probiotic diet at the different exposure period.

No. of Days	Globulin (gm/dl)	
	Control	Experiment
15	0.55±0.25	0.60±0.86
30	1.00±0.50	1.30±0.42
45	1.25±0.28	1.80±0.50
60	1.85±0.86	2.71±0.25

Values are in mean ±SD

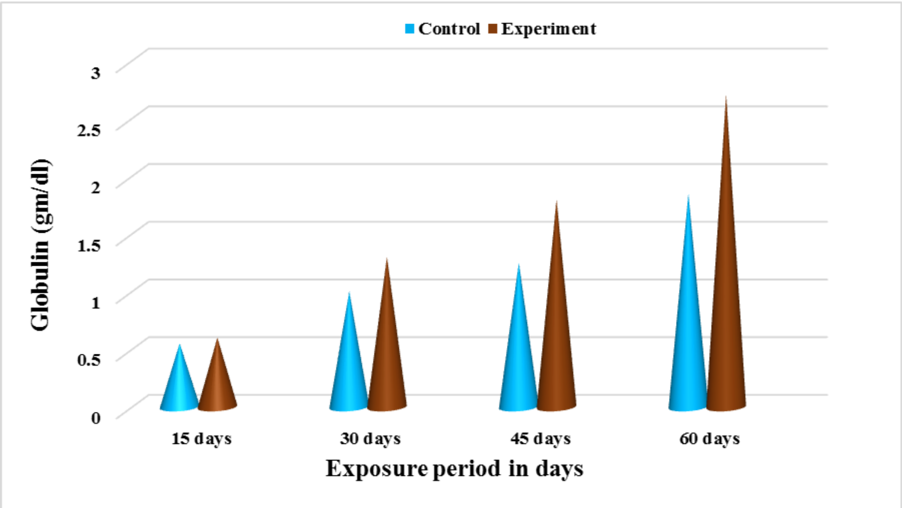

Figure 5: Changes in the globulin of freshwater fish *Labeo rohita* fed with a probiotic diet at the different exposure period.

DISCUSSION

The significances of probiotics are studied in several aquatic animals. Improvement of the expansion has been mentioned by feeding of *Bacillus* species within the *Labeo rohita* (Ghosh *et al*., 2003). The biochemical analyses frequently offer dynamic records for fitness assessment and management of cultured fish (Cnaani *et al*., 2004). The serum glucose is the major carbohydrate exposed in the blood and a chief supply of strength within the human body. The frightened system, appreciate the brain, fully depends on glucose from the encompassing cell fluid (ECF) for power (Michael *et al*., 2005). Serum whole protein and albumin in all trades fed on distinctive tiers of probiotics have higher concentrations than influence whereas globulin showed no tremendous distinction between totally different treatments indicating that probiotics had improved the immunity and extended fitness of the *Labeo rohita*. Cholesterol absorption differs together between and within the fish species because of differences in diet, activity, and sexual development (McDonald & Milligan, 1992). Cholesterol is important for the species growth and possibility of cells in higher in the living organisms. Also, cholesterol is a precursor of steroid hormones such as progesterone, testosterone, oestradiol and cortisol (Aengwanich & Tanomtong *et al*., 2004), but was much lower than data for salmonids (Congleton &Wagner, 2006) rainbow trout (Manera & Britti *et al*., 2006) and may reproduce a species or nutritional difference. The glucose is the major macromolecule found in the blood and a chief supply of energy within the human body. The nervous system, including the brain, totally depends on glucose from the surrounding extracellular fluid (ECF) for energy (Michael *et al*., 2005). The serum total protein and albumin in all groups fed on different levels of probiotics have higher concentrations than control whereas globulin showed no significant difference between different treatment indicating that probiotics had enhanced immunity and improved health of *Labeo rohita*. Fish liver is known to be a high calorific value and an important source of vitamins. The lipid content of the liver was generally high, probably because of its being a centre of fat deposits (Sinnhnber and Law, 1947). From these microorganisms were a particular test against the probiotic properties. It shows the similarity with the findings of other researchers who reported a maximum population of *Bacillus* species was selected in freshwater fish (Dhansekaran *et al*., 2010).

CONCLUSION

The isolated probiotics *bacillus subtilis* were improved growth and biochemical performance in freshwater fish *Labeo rohita*. It can be concluded that the addition of probiotics *Bacillus subtilis* diet improve growth, mitigates and effects of stress factors in fingerlings freshwater fish *Labeo rohita*. The freshwater fish *Labeo rohita* fish used in the present study were effective in stimulating fish growth and biochemical performance, though *Bacillus subtilis* produced the best results, being the most possible option for the beneficial effect of the biochemical parameters and improving growth and feed consumption in intensive freshwater fish *Labeo rohita* culture.

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REFERENCES

1. Aderfemo, O.K. Haematological profile of clariasgariepinus (Burchell, 1822) exposed to lead. *Turkish Journal of Fisheries and Aquatic Sciences*, 2007; 7: 163-169.
2. Aengwanich, W., Tanomtong, A. Hematological and serum biochemical values of white ibis (*Threskiornis melanocephalus*), Songklanakarin. *Journal of Science and Technology*, 2004; 26(6): 823-828.
3. Allain, C.A., Poon, L.S., Chan, C.S.G., Richmond, W., and Paul, C.F. Estimation of cholesterol by enzymatic method. *Clinical Chemistry*, 1974; 20(4): 470-5.
4. Association of Official Analytical Chemists, Official methods of analysis of the association of official analytical chemists. 1(15), Washington DC, 1990; 1134.
5. Capkin, E., Altinok, I. Effects of dietary probiotic supplementations on prevention/treatment of yersiniosis disease. *Journal of Applied Microbiology*, 2009; 106(4): 1147-1153.
6. Cnaani, A., Tinman, S., Avidar, Y., Ron, M., and Hulata, G. Comparative study of biochemical parameters in response to stress in *Oreochromis aureus*, *O. mossambicus* and two strains of *Oreochromis niloticus*. *Aquaculture Research*, 2004; 35(15): 1434-1440.
7. Congleton, J.L., Wagner, T. Blood-chemistry indicators of nutritional status in juvenile salmonids, *Journal of Fish Biology*, 2006; 69: 473-490.
8. Dada, A.A., Fagbenro, O.A., and Fasakin, E. A. Determination of optimum feeding frequency for *Heterobranchus bidorsalis* fry in outdoor concrete tanks. *Journal of Aquaculture in the Tropics*, 2002; 17(3): 167-174.
9. Dhanasekaran, D., Subhasish Saha, N. Thajuddin, M., Rajalakshmi, Panneerselvam, A. Probiotic effect of *Lactobacillus* isolates against bacterial pathogens in fresh water fish. *Journal Coastal Development*, 2010; 13(2): 103–112.
10. FAO/WHO. (2001). Evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria, report of a joint FAO/WHO expert consultation.
11. Ghosh, K., Sen, S.K., and Ray, A.K. Supplementation of an isolated fish gut bacterium, *Bacillus circulans*, in formulated diets for rohu,

Labeo rohita, fingerlings. *Israeii Journal Aquaculture Bamidgeh*, 2003; 55(1): 13–21.
12. Ghosh, S., Sinha, A., and Sahu, C. Dietary probiotic supplementation in growth and health of live-bearing ornamental fishes. *Aquaculture Nutrition*, 2008; 14(4): 289–299.
13. Jayaprakash, S., and Parvathi, K. Influences of isolated gut probiotic *Bacillus subtilis* on growth performance of freshwater fish *Labeo rohita. International Journal of Life sciences Research*, 2019; 7(1): 202-208.
14. Kumar, R., Mukherjee, S.C., Prasad, K.P., and Pal, A.K. Evaluation of *Bacillus subtilis* as a probiotic to Indian major carp *Labeo rohita* (Ham.). *Aquaculture Research*, 2006; 37: 1215-1221.
15. Kumar, V., Makkar, H. P. S., and Becker, K. Nutritional, physiological haematological responses in rainbow trout (*Oncorhynchus mykiss*) juvenile fed detoxified *jatropha curcas* kernel meal. *Aquaculture Nutrition*, 2011; 17: 415-467.
16. Manera, M., & Britti, D. Assessment of blood chemistry normal ranges in rainbow trout (*Oncorhynchus mykiss*), *Journal of Fish Biology*, 2006; 69: 1427-1434.
17. McDonald, D.G., and Milligan, C.L. Chemical properties of the blood. In Hoar, W.S., Randall, D.J., and Farrell, A.P. (eds). *Fish Physiology*, San Diego, *CA*, Academic Press Inc, 12B, 1992; 55-133.
18. Michael, L.Bishop, Edward, P.Fody, Larry Schoeff. Clinical chemistry principles, procedures, and correlations. L.Bishop, Edward P.Fody, Larry Schoeff editors. Fifth edition, Quality control and statistics, 2005; 49-83.
19. Mitruka, B.M., & Rawnsley, H. Clinical, biochemical and haematological references values in normal experimental animals. *Masson Publishing* USA Inc., 1977; 53-54.
20. Pooramini M, Kamali A, Hajimoradloo A, Alizadeh M, Ghorbani R. Effect of using yeast (Saccharomyces cerevisiae) as probiotic on growth parameters, survival and carcass quality in rainbow trout Oncorhynchus mykiss fry. *International Aquatic Research*, 2009; 1(1): 39-44.
21. Schlam, O.W., Jain, N.C., and Carol, E.I.Veterinary Heamatology. 3rd edn. Lea and Fibinger, Philadelphia, 1975; 144 – 167.
22. Sinnhuber, R.O., and Law, D. K. Vitamin A and oil content of fish livers and viscera. Fishes of the Oregon coast. *Industrial and Engineering Chemistry*, 1947; 39: 1309-1310.
23. Sivakumar, P., Rajan, M.R., Ramachandran, P. Effects of probiotics on growth performance of common carp Cyprinus carpio var communis. *International Journal of Pharm Bio Sciences*, 2014; 5(1): 835-839.
24. Tietz, N. Clinical Guide to Laboratory Tests 2nd Ed. Philadelphia, 1990; NLM ID: 101268998. http://dx.doi.org/10.4236/ojm.2013.34020 6,766.
25. Trinder, P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of Clinical Biochemistry*, 1969; 6(24).
26. Whitman, W.B., De Vos, P., Garrity, G.M., Jones, D., Noel, R., Krieg, N.R., Ludwig, W., Rainey, F.A., and Schleifer, K.H. Bergey's manual of systematic bacteriology, 2009; 2(*3).*
27. Young, D. Effects of drugs on clinical laboratory tests. Third Edition, *AACC Press*, Washington, D.C., 1990; 32(3): 30-33.