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Efficacy of Probiotic Bacteria (*Lactobacillus plantarum*) Supplementation in the Diet on Growth and Nutritional Composition of *Cirrhinus mrigala* Fingerlings

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ABSTRACT

Background: The use of probiotics in aquaculture is increasingly acknowledged, thanks to rising demand for environment friendly aquaculture. However, there is an obvious need to improve our understanding of gut microbiology, as well as the effective preparation and safety assessment of probiotics.

Methods: The experiment comprised of five treatments to evaluate the influence of probiotic on water quality, growth and body composition. *L. plantarum* probiotic was sprayed over test diets at various concentrations @ 10^6 to 10^9 CFU/g, (D0 to D4 respectively). Water quality parameters were analysed (APHA, 2012) and growth parameters formulae (Halver, 1957) whereas fish feed and fish flesh nutritional composition, were measured (AOAC, 2012).

Result: All of the water quality parameters were within acceptable limits. Net weight gain (NWG), NWG %, specific growth rate, Condition factor and survival rate were significantly (P<0.05) highest in D4 and lowest in D0. Similarly, Feed conversion ratio and protein efficiency ratio also improved significantly (P<0.05). Moreover, there was a considerable rise in crude protein and lipid content in D4. In general, supplementation of *L. plantarum* @ 10^9cfu/g diet improved growth, survival and nutritional composition of *Cirrhinus mrigala*.

Key words: *Cirrhinus mrigala*, Diet, Growth, *Lactobacillus plantarum*, Nutritional composition.

INTRODUCTION

Aquaculture produces high-quality animal protein, improves nutrition and generates money and employment worldwide. Concerned scientists and policymakers are both optimistic and disturbed by the tremendous increase in the production over the previous two decades. Aquaculture intensification, like any other sector, had a severe influence on the environment, leading to the development of infectious illnesses and other environmental concerns. Economic losses are incurred as a result of disease outbreaks and environmental damage. The use of probiotics, may thus provide plenty of opportunity for fish farming enterprises to expand. Probiotics are currently used in both water and feed in the aquaculture. Several incidences of *Aeromonas hydrophila* infections have been reported in India's Indian major carps in recent years (Shome *et al*. 2005). Increased uses of antimicrobial drugs, pesticides and disinfectants have all been used to combat this, leading in the rise of resistant bacterium strains. As a result, probiotics are becoming increasingly important in the aquaculture business, as the need for environmentally benign, long-term aquaculture grows. The term "probiotics" is derived from the Greek word meaning "life." The term "probiotic" was given by Parker (1974). According to the World Health Organization and the Food and Agriculture Organization, probiotics are the live microorganisms that provide a health benefit to the host when provided in appropriate amounts. Probiotics (live bacteria) that colonise the gut and/or animal-derived microbial supplements, have recently been acknowledged to be advantageous to fish health (Dimitroglou *et al*. 2011). Due to rising demand for environment friendly aquaculture, application of probiotics in aquaculture is increasingly acknowledged. Lactic acid bacteria (LAB) has been widely employed as a food supplement to protect fish from numerous infectious illnesses in recent years (Geng *et al*., 2012). *Lactobacillus* has demonstrated to be effective in fish culture (Barbosa *et al.*, 2011). Because it is resistant to environmental conditions and has a long life cycle, *Lactobacillus* is the most often utilised probiotic in aquaculture and the beneficial activities of these bacterial species in the aquaculture are well known (Wang *et al*., 2008; Kumar *et al*., 2019: Amit *et al*., 2021;

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Amit *et al*., 2022; Totewad and Gyananath, 2021; Gao *et al*., 2016; Sinha and Pandey, 2013).However, there is a clear need to increase our understanding of gut microbiology, as well as the proper dose and safety parameters. Due to its flavour and nutritional quality, *Cirrhinus mrigala* is one of the most significant candidate fish species in India, with the highest market demand and acceptability as food by people. Therefore, the goal of this study was to see how probiotic bacteria (*Lactobacillus plantarum*) affected the survival, growth and body composition of mrigal, *Cirrhinus mrigala*.

MATERIALS AND METHODS

Experimental design

The experiment was conducted at the Fish Farm of Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, in outdoor FRP ponds (1.5 × 1.0 × 0.75 m). The trial included five treatments (Table 1), each with triplicates. Rice bran, mustard meal, vitamin and mineral mixture and salt were used to make the basic diet (49, 49, 1.5 and 0.5 percent respectively). At varied levels (@ 0 (D0), 10^6 CFU/g (D1), 10^7 CFU/g (D2), 10^8 CFU/g (D3) and 10^9 CFU/g (D4), probiotic bacterial strain *L. plantarum* FLB1 was added into basal meals (D0). Fish were stocked @ 15 fingerlings each FRP pool and supplementary diets were supplied *ad libitum* daily for 120 days (May to August).

Experimental diets

The basal diet consisted of de-oiled rice bran (49 percent), mustard meal (49 per cent), vitamin-mineral mixture (1.5 per cent) and salt (0.5 per cent). With the use of an electric lab pelletizer, the basal diet and experimental designed diets for the experiment were made into sinking pellets. *L. plantarum* FLB1 was isolated from the gut of rohu using de Man Rogosa and Sharpe (MRS) agar for the manufacture of experimental diets and the amplified 16S rRNA gene fragment was subjected to Sanger sequencing for genus confirmation in addition to biochemical testing. To determine probiotic qualities, the isolate was examined in vitro for hemolytic activity, acid and bile tolerance for growth kinetics, auto-aggregation, cell-surface hydrophobicity, phenol tolerance, cell adhesion and safety parameters (Zhang *et al*., 2014). *L. plantarum* FLB1 was isolated and the aliquots of *L. plantarum* FLB1 were stored at -80°C in 30% glycerol stock (Kumar et al., 2019; Zhang et al., 2014 at the College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana. For formulations of probiotic diet in the present study, an aliquot of *L. plantarum* FLB1 was removed from -80°C storage and streaked on MRS agar plate. After incubation at 37°C for 48 h, individual colonies were picked up for subculture in MRS broth at 37°C. The cells were extracted by centrifugation (2000g) after incubation to obtain a microbial pellet. The pellet was washed three times in phosphate-buffered saline (pH 7.2), counted and mixed with control diet @10^6(D1), 10^7(D2), 10^8(D3) and 10^9(D4) CFU/g, respectively. The bacterial solution was gently added to feed pellets with a hand sprayer for uniform dispersion in the laminar airflow chamber under sterilized conditions to get correct final concentrations in the diet. The resulting diet was kept in sealed plastic zip lock bags at 4°C until it was utilized. Diets were created fresh once a week to provide a high probiotic level in the supplemented diet. The proximate compositions of the test diets (Table 2), as well as nutritional composition of fish flesh were measured using the Association of Official Analytical Chemists' standard techniques (AOAC, 2012).

Water parameters

Temperature, dissolved oxygen, pH, total alkalinity, nitrate, nitrite and ammonia were all measured every two weeks using APHA's standard techniques (APHA, 2012).

Growth parameters

The following formulae (Halver, 1957) were used to compute total length gain (TLG), net weight gain (NWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and condition factor (K) of fish for each treatment.

TLG= Final total length (TL) (cm)-Initial total length (TL) (cm)

NWG = Final body weight (g)-Initial body weight (g)

$$\text{Weight gain (\%)} = \frac{\text{Final body weight- Initial body weight}}{\text{Initial body weight}} \times 100$$

$$\text{SGR (\% weight gain day}^{-1}) = \frac{ln \text{ final BW (g)} - ln \text{ initial BW (g)}}{\text{Culture days}} \times 100$$

$$\text{Condition factor (K)} = \frac{\text{Body weight (g)}}{\text{Body leng th}^3 \text{ (cm}^3)} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed given (g)}}{\text{Weight gain (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$$

Statistical analysis

The data was statistically analyzed using statistical software (SPSS 20.0, SPSS Inc. and Richmond, CA, USA). Means and standard errors of the mean were used to present the data. The effect of nutritional supplementation with probiotic bacteria (*Lactobacillus plantarum*) on survival, growth and body composition of fish (P<0.5) was determined using one-way ANOVA, followed by Duncan's multiple comparison to determine significant differences among the treatments.

RESULTS AND DISCUSSION

Water quality

Mean water temperature, pH, dissolved oxygen, total alkalinity, ammonia, nitrite and nitrate in different treatments ranged from 30.82 to 30.87°C, 7.92 to 7.99, 7.79 to 7.91

Table 1: Composition of experimental diets.

Treatments				
Control Diet	A basal diet supplemented with gut probiotic bacterial culture			
D0	D1	D2	D3	D4
Basal diet*	Basal diet+*L. plantarum* @ 10^6CFU/g feed	Basal diet+*L. plantarum* @ 10^7 CFU/g feed	Basal diet+*L. plantarum* @ 10^8 CFU/g feed	Basal diet+*L. plantarum* @ 10^9 CFU/g feed

*Rice bran (49%) + Mustard meal (49%) + Vit-Min. mixture (1.5 %) + Salt (0.5%).

Table 2: Proximate composition (on % dry matter basis) of feed ingredients and experimental diets used in experiment.

Ingredients/ Experimental diets	Crude protein	Ether extract	Crude fiber	Ash	NFE
Rice Bran*	13.83	1.27	15.69	11.32	57.89
Mustard meal*	39.94	1.97	11.12	7.23	39.74
		Experimental diets			
D0	26.82	4.02	17.60	7.82	43.75
D1	26.95	4.06	17.41	7.79	43.79
D2	27.07	4.08	17.37	7.67	43.80
D3	27.21	4.13	17.36	7.72	43.59
D4	27.76	4.20	17.35	7.72	42.97

*Solvent extracted.

mg/l, 219.96 to 228.81 mg/l, 0.054 to 0.061 mg/l, 0.048 to 0.053 mg/l and 0.25 to 0.31 mg/l respectively. The water quality parameters showed no significant differences (P<0.05) and remained well within the optimum rage for carps (Table 3).

Effect of diet supplementation with *L. plantarum* on growth and survival

D4 had the greatest values (29.00, 214.37, 0.95 and 1.12) of NWG (g), percent NWG, SGR and condition factor (K) respectively (Table 4) while D0 had the lowest values (17.50, 126.94, 0.68 and 1.07) respectively at the end of the study period (120 days). At the end of the trial, the survival rate (percentage) in all probiotic fed treatments ranged from 91.11% to 97.78%, indicating that probiotic supplemented diet had no negative effects. Survival (per cent) improved in probiotic-incorporated diets (D1-D4), with a significant (P<0.05) improvement in D4, when compared to control (D0), PER and FCR also improved considerably (1.96 and 1.84, respectively) in D4 (Table 5).

Effect of diet supplementation with *L. plantarum* on nutritional composition of fish

Fish flesh quality (wet weight basis) in terms of crude proteins, lipid, ash, total carbohydrate and moisture content was estimated at the end of the experiment and the results are provided in Table 6. In all treatments (D0 to D4), the crude protein content in the fish meat ranged from 13.90 to 14.63%. The crude protein content of treatments D0, D1, D2, D3 and D4 was 13.90, 13.97, 14.11, 14.35 and 14.63 percent, respectively. The fish had a crude protein content of 13.66 percent at the time of stocking. The variations in protein content between the treatments were significant (P<0.05) (D4>D3>D2>D1=D0>Initial). Probiotic in the diet significantly (P<0.05) improved meat quality in terms of crude protein content when compared to the control (D0) and time of stocking. In all treatments (D0 to D4), the total lipid content in the fish flesh varied from 2.22 to 3.10 g 100/g. Treatments D0, D1, D2, D3 and D4 had lipid content of 2.22, 2.51, 2.74, 2.91 and 3.10% respectively. Fish had a lipid content of 1.92 when they were stocked. There were considerable differences in lipid content amongst the treatments (D4>D3>D2>D1>D0>Initial). As a result, probiotic improved nutritional quality in terms of lipid content considerably (P<0.05). When compared to the control group, all probiotic fed groups demonstrated a substantial (P<0.05) increase in *C. mrigala* meat crude protein and lipid content as well as a significant (P<0.05) decrease in total ash, moisture and total carbohydrate content compared to the control (D0) in the present study. Overall, inclusion of probiotics improved nutritional quality of the fish.

However, based on growth and immunological parameters, there is limited information on the integration of probiotics into carp cultures (Chi *et al*., 2014; Gupta *et al*., 2014; Kumar *et al*., 2019: Amit *et al*., 2021; Amit *et al*., 2022; Totewad and Gyananath, 2021; Gao *et al*., 2016; Sinha and Pandey, 2013), particularly *Cirrhinus mrigala*. The majority of study in this field, namely probiotic supplementation in fish, has been focused on species such as trout, tilapia and catfish, as well as shellfish (shrimp, prawns and oyster). Probiotics are typically described as multifunctional and can be used on a variety of species under a variety of growth conditions. Apart from these advantages, probiotics are more effective at converting organic matter to CO_2, hence high doses of probiotics in production ponds are advised (Wang and Wang, 2008). When fingerlings of *Cyprinus carpio* were fed *L. plantarum* @ 4.5×10^6 CFU/mg, Valiallahi *et al*. (2018) observed an enhanced survival rate, specific growth rate and reduced FCR. Gupta et al. (2014) observed that feeding

Bacillus coagulans, *B. licheniformis* and *P. polymyxa* 10^9/g incorporated food to *Cyprinus carpio* resulted in improved growth, a minimised feed conversion ratio and an enhanced protein efficiency ratio. Abumourad *et al*. (2013) found similar results in *O. niloticus* and Enferadi *et al*. (2018) found similar outcomes in *O. mykiss* when fed *L. plantarum*.

Furthermore, differences in the condition factor (K) of fish between treatments were significant when compared to the control, suggesting that probiotic incorporation had no negative impact on the condition of fish, which is used to measure the health of fish and indicates proportional weight gain with length gain (Nash *et al*. 2006).

When compared to control, all probiotic fed groups showed better protein and lipid in *Cirrhinus mrigala* meat, whereas D4 had lower ash, moisture and carbohydrate values in this study. Overall, the results demonstrated that supplementing the diet with probiotics improved the nutritional quality of the mrigal flesh with probiotic treatment

Table 3: Water quality parameters in different treatments during experiment*.

Parameters	Treatments (with different levels of *L. plantarum*)				
	D0	D1	D2	D3	D4
Water temperature (°C)	$30.83^{a}\pm0.48$	$30.87^{a}\pm0.49$	$30.83^{a}\pm0.47$	$30.86^{a}\pm0.50$	$30.82^{a}\pm0.51$
pH	$7.94^{d}\pm0.11$	$7.95^{c}\pm0.10$	$7.92^{e}\pm0.11$	$7.98^{b}\pm0.11$	$7.99^{a}\pm0.09$
Dissolved oxygen (mg/l)	$7.91^{a}\pm0.11$	$7.80^{c}\pm0.12$	$7.79^{cd}\pm0.14$	$7.85^{b}\pm0.13$	$7.90^{ab}\pm0.10$
Total alkalinity (mg/l)	$219.96^{d}\pm1.65$	$225.14^{c}\pm1.18$	$227.66^{b}\pm1.63$	$228.81^{a}\pm1.79$	$227.32^{b}\pm1.53$
Ammonia nitrogen (mg/l)	$0.055^{d}\pm0.003$	$0.059^{b}\pm0.006$	$0.057^{c}\pm0.005$	$0.054^{e}\pm0.006$	$0.061^{a}\pm0.004$
Nitrite nitrogen (mg/l)	$0.050^{b}\pm0.003$	$0.048^{d}\pm0.003$	$0.053^{a}\pm0.002$	$0.053^{a}\pm0.002$	$0.049^{c}\pm0.003$
Nitrate nitrogen (mg/l)	$0.28^{b}\pm0.042$	$0.25^{cd}\pm0.039$	$0.26^{c}\pm0.033$	$0.30^{ab}\pm0.041$	$0.31^{a}\pm0.042$

*Values (Mean±S.E.) with different alphabetical superscripts in a row differ significantly (P<0.05).

Table 4: Growth and survival of *Cirrhinus mrigala* in different treatments during the experimental period*.

Parameters	Treatment				
	D0	D1	D2	D3	D4
TLG	$2.49^{c}\pm0.03$	$2.80^{c}\pm0.16$	$3.21^{b}\pm0.20$	$3.52^{ab}\pm0.09$	$3.77^{a}\pm0.03$
TLG%	$21.13^{d}\pm0.27$	$23.83^{cd}\pm1.70$	$27.36^{bc}\pm2.14$	$30.20^{ab}\pm0.87$	$31.86^{a}\pm0.17$
NWG (g)	$17.50^{e}\pm0.51$	$19.61^{d}\pm0.12$	$23.75^{c}\pm0.14$	$26.26^{b}\pm0.12$	$29.00^{a}\pm0.19$
NWG (%)	$126.94^{d}\pm4.18$	$141.62^{c}\pm2.66$	$183.34^{b}\pm3.66$	$205.77^{a}\pm0.59$	$214.37^{a}\pm1.87$
SGR	$0.68^{d}\pm0.02$	$0.73^{c}\pm0.01$	$0.87^{b}\pm0.01$	$0.93^{a}\pm0.00$	$0.95^{a}\pm0.01$
Condition factor (K)	$1.07^{b}\pm0.01$	$1.08^{b}\pm0.00$	$1.10^{ab}\pm0.01$	$1.11^{ab}\pm0.02$	$1.12^{a}\pm0.01$
Survival rate (%)	$82.22^{b}\pm2.22$	$91.11^{a}\pm2.22$	$93.33^{a}\pm0.00$	$95.56^{a}\pm2.22$	$97.78^{a}\pm2.22$

*Values (Mean±S.E.) with different alphabetical superscripts in a row differ significantly (P<0.05).

TLG= Total length gain, NWG= Net weight gain, SGR= Specific growth rate.

Table 5: Feed efficiency of *Cirrhinus mrigala* in different treatments during the experimental period*.

Parameters	Treatment				
	D0	D1	D2	D3	D4
PER	$1.31^{e}\pm0.04$	$1.44^{d}\pm0.01$	$1.69^{c}\pm0.01$	$1.84^{b}\pm0.01$	$1.96^{a}\pm0.01$
FCR	$2.85^{a}\pm0.08$	$2.58^{b}\pm0.02$	$2.19^{c}\pm0.01$	$2.00^{d}\pm0.00$	$1.84^{e}\pm0.01$

*Values (Mean±S.E.) with different alphabetical superscripts in a row differ significantly (P<0.05).

FCR= Feed conversion ratio, PER= Protein efficiency ratio.

Table 6: Nutritional composition (wet weight basis) of *Cirrhinus mrigala* in different treatments*.

Parameter (%)	Treatment					
	Initial	D0	D1	D2	D3	D4
Moisture	$79.50^{a}\pm0.11$	$79.48^{a}\pm0.11$	$79.25^{b}\pm0.01$	$79.10^{bc}\pm0.03$	$79.03^{c}\pm0.05$	$78.66^{d}\pm0.02$
Total proteins	$13.66^{e}\pm0.06$	$13.90^{d}\pm0.05$	$13.97^{d}\pm0.01$	$14.11^{c}\pm0.03$	$14.35^{b}\pm0.02$	$14.63^{a}\pm0.01$
Total lipid	$1.92^{f}\pm0.05$	$2.22^{e}\pm0.06$	$2.51^{d}\pm0.01$	$2.74^{c}\pm0.02$	$2.91^{b}\pm0.03$	$3.10^{a}\pm0.04$
Total carbohydrate	$2.76^{a}\pm0.03$	$2.25^{b}\pm0.02$	$2.19^{b}\pm0.02$	$1.99^{c}\pm0.02$	$1.73^{d}\pm0.03$	$1.74^{d}\pm0.03$
Ash	$2.16^{a}\pm$ 0.02	$2.14^{ab}\pm0.00$	$2.08^{bc}\pm0.03$	$2.05^{c}\pm0.03$	$1.98^{d}\pm0.03$	$1.87^{e}\pm0.02$

*Values (Mean±S.E.) with different alphabetical superscripts in a row differ significantly (P<0.05).

could be related to increased fish growth, as seen in this study and in a previous investigation (Amit *et al*. 2021; Ghosh *et al*. 2003; Giri *et al*. 2013). Improved protein efficiency ratio may have also aided in the improvement of fish flesh quality (improved PER in D4). Probiotics also increased feed efficiency, growth and improved health parameters in fish, according to Valiallahi *et al*. (2018). Adding probiotics to fish's diets improved the efficiency of converting feed protein to flesh Gatesoupe, 1999). Probiotics have been found to eliminate anti-nutritional components in feeds, resulting in improved nutritional quality and bioavailability as seen by higher growth and nutritional composition.

CONCLUSION

It can be concluded that *L. plantarum* boosted feed efficacy and protein deposition in fish flesh as well. Overall, adding probiotics to the diet improved water quality, fish's growth and nutritional quality in a synergistic way. As a result, incorporating *L. plantarum*, in fish feed improved survival, growth, feed efficacy, K-value and nutritional quality of mrigal fingerlings significantly (P<0.05), with the best results in D4 (@10^9 CFU/g feed). Hence, it can be added to the mrigal's diet during the growth period of the fish in a semi-intensive culture system. With the drive for environment friendly sustainable aquaculture, probiotics have become an integral element of the process. The information gathered in this study will aid in boosting fish production and productivity in a sustainable manner, as well as raising fish farmers' revenue and contributing to the nation's nutritional security.

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REFERENCES

Abumourad, I.M.K., Abbas, W.T., Awaad, E.S., Authman, M.M.N., El-Shafei, K., Ibrahim, O.M.S., Gamal, A., Sadek, Z.I., El-Sayed, H.S. (2013). Evaluation of *Lactobacillus plantarum* as a probiotic in aquaculture: Emphasis on growth performance and innate immunity. Journal of Applied Sciences Research. 9(1): 572-582.

Amit, Pandey, A., Khairnar, S.O., Tyagi, A. (2021). Effect of Dietary Supplementation of Probiotic Bacteria (*Lactobacillus plantarum*) on Growth and Proximate Composition of *Cyprinus carpio* Fingerlings. National Academy Science Letters-India. 44: 495-502.

Amit, Pandey, A., Tyagi, A., Khairnar, S.O. (2022). Oral feed-based administration of *Lactobacillus plantarum* enhances growth, haematological and immunological responses in *Cyprinus carpio* ", Emerging Animal Species. 3: 100003.

AOAC. (2012). Official Methods of Analysis of AOAC International. 19th edition. AOAC 54 International, Gaithersburg, Maryland, USA.

APHA. (2012). Standard Methods for the Examination of Water and Wastewater. 22nd Edn. American Public Health Association, Washington, D.C. 1360 p.

Barbosa, M.C., Jatobá, A., Vieira, F.N., Silva, B.C., Mourino, J.L., and andreatta, P.E.R., Seiffert, W.Q., Cerqueira, V.R. (2011). Cultivation of juvenile fat snook (*Centropomus parallelus* Poey, 1860) fed probiotic in laboratory conditions. Brazilian Archives of Biology and Technology. 54 (4): 795-801.

Chi, C., Jiang, B., Yu, X.B., Liu, T.Q., Xia, L., Wang, G.X. (2014). Effects of three strains of intestinal autochthonous bacteria and their extracellular products on the immune response and disease resistance of common carp, *Cyprinus carpio*. Fish and Shellfish Immunology. 36 (1):9-18.

Dimitroglou, A., Merrifield, D.L., Carnevali, O., Picchietti, S., Avella, M., Daniels, C., Guroy, D., Davies, S.J. (2011). Microbial manipulations to improve fish health and production-A Mediterranean perspective. Fish and Shellfish Immunology. 30:1-16.

Enferadi, M.H.N., Mohammadizadeh, F., Soltani, M., Bahri, A.H., Sheikhzadeh, N. (2018). Effects of *Lactobacillus plantarum* on growth performance, proteolytic enzymes activity and intestine morphology in rainbow trout (*Oncorhynchus mykiss*). Turkish Journal of Fisheries and Aquatic Sciences. 18: 351-356.

Gao, Q., Xiao, C., Min, M., Zhang, C., Peng, S., Shi, Z. (2016). Effects of probiotics dietary supplementation on growth performance, innate immunity and digestive enzymes of silver pomfret, *Pampus argenteus*. Indian Journal of Animal Research. 1-6.

Gatesoupe, F.J. (1999). The use of probiotics in aquaculture. Aquaculture. 180: 147-165.

Geng, X., Dong, X.H., Tan, B.P., Yang, Q.H., Chi, S.Y., Liu, H.Y., Liu, X.Q. (2012). Effects of dietary probiotic on the growth performance, non-specific immunity and disease resistance of cobia, *Rachycentron canadum*. Aquaculture Nutrition. 18 (1): 46 55.

Ghosh, K., Sen, S.K., Ray, A.K. (2003). Supplementation of an isolated fish gut bacterium, *Bacillus circulans*, in formulated diets for rohu, *Labeo rohita* fingerlings. Israeli Journal of Aquaculture-Bamidgeh. 55: 13-21.

Giri, S.S., Sukumaran, V., Oviya, M. (2013). Potential probiotic *Lactobacillus plantarum* VSG3 improves the growth, immunity, and and disease resistance of tropical freshwater fish, *Labeo rohita*. Fish and Shellfish Immunology. 34(2): 660-666.

Gupta, A., Gupta, P., Dhawan, A. (2014). Dietary supplementation of probiotics affects growth, immune response and disease resistance of *Cyprinus carpio* fry. Fish and Shellfish Immunology. 41: 113-119.

Halver, J.E. (1957). Nutrition of salmonoid fishes. III. Water-soluble vitamin requirements of chinook salmon. The Journal of Nutrition. 62: 225-243.

Kumar, P., Kaur, V.I., Tyagi, A., Nayyar, S. (2019). Probiotic Potential of Putative Lactic Acid Bacteria Isolated from the Fish Gut: Immune Modulation in *Labeo rohita* (Ham.). Journal of Coastal Research. 86(SI): 119-127.

Nash, R.D.M., Valencia, A.H., Geffen, A. (2006). The origin of Fulton's condition factor-Setting the record straight. Fisheries. 31: 236-238.

Parker, R.B. (1974). Probiotics the other half of the antibiotic story. Animal Nutrition and Health. 29: 4-8.

Shome, R., Shome, B.R., Mazumdar, Y., Das, A., Kumar, A. (2005). Abdominal dropsy disease in major carps of Meghalaya: isolation and characterization of *Aeromonas hydrophila*. Current Science of India. 88: 25.

Sinha, A., Pandey, P.K. (2013). Probiotic effect of a live bacterial isolate in nutrition of an Indian major carp, rohu (*Labeo rohita*). Indian Journal of Animal Research. 47 (6) : 509-514.

Totewad, N.D. and Gyananath, G. (2021). Effect of Probiotic *Enterococcus gallinarum* N3 Supplemented Feed on Growth Performance of Freshwater Fish Cyprinus Carpio. Indian Journal of Animal Research. DOI: 10.18805/ag.D-5322.

Valiallahi, J., Pourabasali, M., Janalizadeh, E., Biucio, A. (2018). Use of *Lactobacillus* for improved growth, and and enhanced biochemical, hematological and digestive enzyme activity in fishes, (*Cyprinus carpio* L.) at Mazandaran Iran. North American Journal of Aquaculture. 80 (2): 206-215.

Wang, Y.M. and Wang, Y.G. (2008). Advance in the mechanisms and application of microecologics in aquaculture. Progress in Veterinary Medicine. 29: 72-75.

Zhang, J., Zhang, X., Zhang, L., Zhao, Y., Niu, C., Yang, Z., Li, S. (2014). Potential probiotic characterization of *Lactobacillus plantarum* strains isolated from Inner Mongolia "Hurood" cheese. Journal of Microbiology and Biotechnology. 24(2): 225-235.