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Effect of *Lactobacillus rhamnosus* GG Supplementation on the Growth Performance, Survival rate and Morphometry of Grass Carp (*Ctenopharyngodon idella*)

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Authors' Contribution
MZA designed the study and supervised the research work. MF and MJ performed the experiment and compile the data. MSA and SR helped in experimental work. ZM and MQK analyzed the data and guided the manuscript writing. MF processed the data and wrote manuscript.

Key words
Grass carp, Probiotics, *L. rhamnosus* GG, Growth performance, Morphometry

ABSTRACT

The current study was conducted to assess the impact of market available probiotic containing *Lactobacillus rhamnosus* GG bacteria on the growth performance and morphometric characters of grass carp for 60 days. A total of 120 fish fingerlings of mean weight 2.95±0.27g were randomly distributed into four experimental groups having three replicates (n=10/aquarium) and fed with four experimental diets i.e., D1 (commercial fish feed without probiotics as control), D2, D3 and D4 (commercial fish feed with addition of 2g, 4g and 6g *Lactobacillus rhamnosus* GG/ kg respectively) @ 5% of their body weight. Growth performance was determined in terms of final body weight (FBW), weight gain (WG), percent weight gain (%WG), average daily weight gain (AWG), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (SR) of fingerlings. Morphometric traits were measured including total length (TL), standard length (SL), fork length (FL), head length (HL), eye diameter (ED), dorsal fin length (DFL), pectoral fin length (PFL), pelvic fin length (PvFL), anal fin length (AFL) and caudal fin length (CFL). The highest FBW (14.20±0.08g), WG (11.25±0.08g), AWG (0.18±0.00g), SGR (2.61±0.05% day^{-1}), %WG (381.340±14.40g), and SR (100%) and lowest FCR (1.94±0.00) were recorded in D4 ($P< 0.05$) followed by D3, D2 and D1. Fish fed with probiotics showed improved ($P<0.05$) morphometric traits and highest TL(15.19±0.29cm), SL(13.17±0.40cm), FL(14.04±0.28cm), HL(1.76±0.04cm), ED(0.62±0.04cm), DFL(1.82±0.04cm), PFL(1.43±0.04cm), PvFL(1.36±0.05cm), AFL(1.41±0.09cm) and CFL(1.82±0.04cm) was observed in D4. The result of this study demonstrated the importance of commercial probiotic as feed additives containing *L. rhamnosus* GG to improve the growth, survival rate and morphometric parameters of grass carp.

INTRODUCTION

Aquaculture industry has become one of the rapidly developing food production sectors (FAO, 2020), contributing to 47 percent of global fish production (FAO, 2018). In recent years, fish production from aquaculture sector increased to 70 million tons (FAO, 2018) that supply high quality animal protein and provide revenue and jobs around the world. Asia significantly contributes to global aquaculture production of farmed aquatic animals (FAO, 2020) and Pakistan export 10 percent of whole capture (Ullah *et al*., 2018). Grass carp is natural inhabitant of rivers and lakes in eastern Asia and introduced into Pakistan in 1964 (FAO, 1970). It is widely cultivated fresh water fish species in the world due to its rich nutritional value, high rate of growth and affordable price. In 2018, 5704 thousand tons of grass carp was produced through aquaculture that accounts for 11% of global fish culture production (FAO, 2018).

With increasing commercialization and aquaculture yield, disease outbreaks and environmental degradation are major barriers to sustainable fish production and cause huge economic losses (Bondad-Reantaso *et al*., 2005). Fish sensitivity to numerous pathogenic organisms enhanced

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by increasing intensity of fish farming, which creates environmental stress that can substantially limit growth and cause infectious illness in farmed fish (Fan *et al*., 2018). The use of antibiotics, antimicrobials and chemotherapy in aquaculture disease control and growth enhancement results in the formation of resistant pathogenic bacterial strains which has decreased their effectiveness for animals (Pandey *et al*., 2022; Lee *et al*., 2016). However, residual antibiotics in aquaculture products may show some side effects on human health and aquatic environment (Cabello, 2006; WHO, 2006).

Probiotics are considered to be safe alternative to antibiotics which are environment friendly and successfully implemented as feed additives in fish farming. Probiotics are the live microbiota used as a feed additive which when ingested by the host, increase the stability of intestinal microbes and therefore improves the growth and resistance against pathogens of the host organism (Dimitroglou *et al*., 2011). Probiotics enhance digestion process by releasing enzymes, better nutritional values, inhibit pathogenic activity, antitumor process and act as immune modulators for host (Lara-Flores *et al*., 2003; Wang *et al*., 2007). According to WHO and FAO, living microbes that impart beneficial effects on the host health when taken in sufficient quantity are probiotics. Currently all types of probiotics belonging to genera *Bacillus, Lactobacillus, Enterococcus, Carnobacterium*, and yeast has extensively applied in aquaculture system (Van Doan *et al*., 2019). Probiotics provided to the host either by addition to supplementary feed or in culture water (Carnevali *et al*., 2014).

Lactic acid bacteria are beneficial flora prevalent in the gut, actively involved in formation of antimicrobials substance, enhanced immune response, greater fish resistance to bacterial infections, utilization of some indigestible carbohydrates and increased accessibility of nutrients, (Fuller, 1989; Nikoskelainen *et al*., 2001). In recent years, it has been commonly used dietary additive to prevent fish from a variety of infectious diseases (Geng *et al*., 2012) and found to be very efficient in fish culture. Several human probiotics has been successfully implemented for aquatic animals demonstrating positive impact on growth performance and resistance against diseases (Pirarat *et al*., 2015). *Lactobacillus rhamnosus* strains administered as probiotics in many trials enhance the hosts growth rate and gastrointestinal defense (Klopper *et al*., 2018; Miyauchi *et al*., 2009; Sewaka *et al*., 2019). Proper dose of probiotics as feed additive is very essential to get better results. There is no study reported regarding the effect of *Lactobacillus rhamnosus* GG on the growth rate and morphometry of grass carp. Therefore, current study was conducted to evaluate the impact of different doses of *L. rhamnosus* GG on the growth and morphometric parameters of grass carp.

MATERIALS AND METHODS

Experimental site

The experimental research was conducted at Aquaculture and Fisheries Laboratory, Department of Zoology, Wildlife and Fisheries, Faculty of Sciences, PMAS-Arid Agriculture University Rawalpindi.

Sample collection

Grass carp fingerlings of initial mean weight of 2.95±0.27g and mean initial values of morphometric parameters including TL 6.00±0.32cm, FL 5.37±0.24cm, SL 4.8±0.22cm, HL 1.34±0.04cm, ED 0.44±0.04cm, DFL 1.03±0.07cm PFL 0.83±0.06cm, PvFL 0.64±0.05cm, AFL 0.74±0.05cm, and CFL 1.24±0.05 cm were acquired from commercial fish seed hatchery and conveyed to the aquaculture and fisheries laboratory in polythene bags filled with water and oxygen.

Preparation of experimental diet

Commercial fish feed pellets (1.5mm) containing 30% crude protein (Marine Grow Fish Feed; Hi-Tech Feeds Private Limited, Pakistan) as a basal diet and market available probiotic bacteria (PREPRO) having *L. rhamnosus* GG was used to prepare four different experimental diets. Basal diet was used as control (D1) without probiotics. Experimental diets D2, D3 and D4 were prepared with addition of 2g, 4g and 6g *L. rhamnosus* GG /kg of basal diet respectively as probiotics.

Study design

The acquired grass carp fingerlings were acclimatized to laboratory conditions using basal diet for 10 days in glass aquaria having the size of 1 x 1x 1.5 feet (length x width x height). After acclimatization, total 120 fish fingerlings were randomly distributed into four experimental groups having three replicates with the stocking density of 10 fish fingerlings per aquarium. Oxygen was supplied to fish using air stone aerators powered by electricity. The fish were fed twice a day at the rate of 5% of their body weight for 60 days. The leftover feed and feces were siphoned out and 50% aquarium water was changed daily for maintenance of water quality.

Assessment of growth performance, feed utilization and survival rate

Growth performance was assessed in terms of final body weight (FBW), weight gain (WG), specific growth rate (SGR), average daily weight gain (AWG), percent weight gain (%WG), feed conversion ratio (FCR) and survival rate (SR). Each fish was weighed fortnightly by using electronic balance. Fish weight was used to adjust the feeding rate throughout the experimental time period. Growth parameters were calculated by using equation described by El-Kady *et al*. (2022) and Kanwal and Tayyab (2019).

Weight gain (WG; g) = Final body weight(g)– initial body weight(g)

Average daily weight gain (AWG; g/day) = Final body weight(g) – initial body weight(g) /Number of days

Percent weight gain (WG%) = Final body weight(g)– initial body weight(g)/initial body weight(g)×100

Specific growth rate (SGR; %/day) = ln final body weight(g)– ln initial body weight(g)/ days×100

Feed conversion ratio (FCR) = Total feed given(g)/ body weight gain(g)

Survival rate (SR; %) = Number of fish survived/ total number of fish×100

Measurements of morphometric characters

A total of 10 morphometric characters of fish were measured using procedure described by Khalid and Naeem (2017). The morphometric parameters of fish were measuring by total length (TL), standard length (SL), fork length (FL), head length (HL), eye diameter (ED), dorsal fin length (DFL), pectoral fin length (PFL), pelvic fin length (PvFL), anal fin length (AFL) and caudal fin length (CFL). For this purpose, 5 fish individuals were taken from each aquarium randomly and anesthetized them with clove oil. Each fish was weighed separately by using weighing balance, the morphometric parameters were measured by using measuring scale closest to 0.1cm and released back into their respective aquarium. The TL was measured from start of snout to the end of caudal fin blade, FL from snout to the bifurcation of caudal fin and SL from the snout tip to start of caudal fin blade. HL measured from snout tip to back end of operculum and ED measured by the space between edges of the eye ball. DFL, PvFL, AFL, PFL and CFL were measured by their longest fin rays from base.

Statistical analysis

The growth and morphometric parameters analysis was done by one-way analysis of variance (ANOVA) with Duncan's Multiple Range Test (DMRT) by using SPSS software (version 16.0). A Pearson correlation test was used to identify correlation between final body weight and morphometric traits. The obtained results were presented as means ± standard deviation (SD) and at the significance level of P<0.05.

RESULTS

Growth performance, feed utilization and survival rate

L. rhamnosus GG administered in the diet of grass carp fingerlings improved the growth parameters of fish in terms of FBW, WG, AWG, SGR, %WG, FCR, and SR (Table I). Probiotic treated groups showed improved growth performance (P < 0.05) than control group. WG, AWG, %WG and SGR significantly enhanced (P < 0.05) in the D4 group treated with 6g of probiotic per kg of basal diet followed by D3, D2 and D1 group. Feed conversion ratio (FCR) improved in dietary groups supplemented with probiotic. Lowest FCR (P < 0.05) was observed in D4 than D1, D2 and D3. The survival rate was recorded 100% in all groups.

Table I. Growth Performance, feed utilization and survival rate (Means ± SD) of grass carp (*Ctenopharyngodon idella*) fingerlings fed with different experimental diets.

Growth parameters	D1	D2	D3	D4
IBW(g)	2.93±0.08	2.98±0.07	2.95±0.07	2.95±0.07
FBW(g)	9.67±0.08^{d}	11.88±0.07^{c}	12.34±0.10^{b}	14.20±0.08^{a}
WG (g)	6.74±0.07^{d}	8.90±0.05^{c}	9.39±0.12^{b}	11.25±0.08^{a}
AWG (g/day)	0.11±0.00^{d}	0.14±0.00^{c}	0.15±0.00^{b}	0.18±0.00^{a}
WG (%)	229.82±5.44^{d}	298.89±3.08^{c}	318.09±6.25^{b}	381.340±14.40^{a}
SGR (%/day)	1.98±0.02^{d}	2.30±0.01^{c}	2.37±0.02^{b}	2.61±0.05^{a}
FCR	2.42±0.02^{a}	2.04±0.00^{b}	2.03±0.04^{b}	1.94±0.00^{c}
SR(%)	100	100	100	100

Mean in the same row with different superscripts a, b and c are significantly different (P < 0.05). IBM, average initial body weight; FBW, average final body weight; WG, weight gain; AWG, average daily weight gain; WG (%), percent weight gain; SGR, specific growth rate; SR, survival rate. D1, basal diet; D2, basal diet+2g *L. rhamnosus*/kg; D3, basal diet+4g *L. rhamnosus*/kg; D4, basal diet+6g *L. rhamnosus*/kg. Data is presented as mean ± standard deviation.

Fortnightly weight gain

Fortnightly weight gain in D4 was significantly higher than D1, D2 and D3 ($P < 0.05$). All probiotics fed groups showed significant increase in weight gain than control as shown in Figure 1.

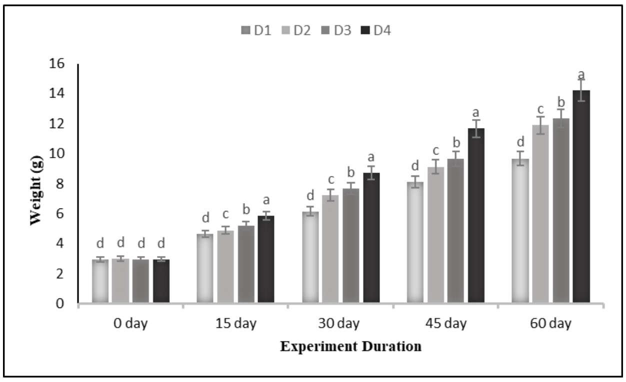

Fig. 1. Effect of different experimental diets on the fortnightly weight gain of grass carp.
The different superscripts on the bars show significant differences among the dietary groups ($P<0.05$) and same superscripts represent non-significant difference among groups ($P>0.05$).

Table II. Effect of different experimental diets on the morphometric traits (Means±SD) of grass carp fingerlings.

Morpho-metric parame-ters	D1	D2	D3	D4
TL	10.78 ± 0.33^{d}	12.94 ± 0.38^{c}	13.44 ± 0.42^{b}	15.19 ± 0.29^{a}
FL	9.72 ± 0.30^{d}	12.00 ± 0.35^{c}	12.45 ± 0.38^{b}	14.04 ± 0.28^{a}
SL	9.07 ± 0.36^{d}	11.35 ± 0.43^{c}	11.71 ± 0.37^{b}	13.17 ± 0.40^{a}
HL	1.62 ± 0.04^{c}	1.72 ± 0.05^{b}	1.72 ± 0.07^{ab}	1.76 ± 0.04^{a}
ED	0.52 ± 0.04^{b}	0.53 ± 0.04^{b}	0.55 ± 0.05^{b}	0.62 ± 0.04^{a}
DFL	1.61 ± 0.05^{c}	1.71 ± 0.03^{b}	1.73 ± 0.04^{b}	1.82 ± 0.04^{a}
PFL	1.30 ± 0.06^{c}	1.34 ± 0.06^{bc}	1.37 ± 0.07^{b}	1.43 ± 0.04^{a}
PvFL	1.15 ± 0.05^{c}	1.24 ± 0.05^{b}	1.28 ± 0.05^{b}	1.36 ± 0.05^{a}
AFL	1.23 ± 0.04^{c}	1.30 ± 0.07^{b}	1.34 ± 0.05^{b}	1.41 ± 0.09^{a}
CFL	1.66 ± 0.04^{c}	1.72 ± 0.05^{b}	1.76 ± 0.05^{b}	1.82 ± 0.04^{a}

For detail of dietary groups and statistical analysis, see caption of Table I. TL, total length; FL, fork length; SL, standard length; HL, head length; ED, eye diameter, DFL, dorsal fin length; PFL, pectoral fin length; PvFL, pelvic fin length; AFL, anal fin length; CFL, causal fin length. All morphometric parameters are measured in centimeters (cm).

Morphometric parameters

The TL, FL, SL, HL, ED, DFL, PFL, PvFL, AFL and CFL were significantly enhanced in the D4 ($P<0.05$) than D2, D3 and D1. DFL, PvFL, AFL and CFL in D2 and D3 did not significantly differ ($P>0.05$) from each other. There was no significant ($P>0.05$) difference in eye diameter and PFL of D1, D2 and D3. HL of D2, D3 and D4 differ non-significantly ($P>0.05$) to each other presented in Table II. Morphometric traits were positive correlated with final body weight in all groups fed with various experimental diets presented in Table III.

Table III. Weight-lengths correlation of grass carp (*Ctenopharyngodon idella*) fed with different experimental diets.

Parame-ters	D1		D2		D3		D4	
	r	p	r	p	r	p	r	p
W-TL	0.970	0.00	0.686	0.00	0.846	0.00	0.942	0.00
W-FL	0.937	0.00	0.598	0.01	0.848	0.00	0.898	0.00
W-SL	0.927	0.00	0.755	0.00	0.720	0.00	0.590	0.02
W-HL	0.878	0.00	0.612	0.01	0.515	0.01	0.820	0.00
W-ED	0.753	0.00	0.814	0.00	0.851	0.00	0.590	0.02
W-DFL	0.756	0.00	0.628	0.01	0.656	0.00	0.756	0.00
W-PFL	0.740	0.00	0.746	0.00	0.911	0.00	0.791	0.00
W-PvFL	0.781	0.00	0.874	0.00	0.853	0.00	0.873	0.00
W-AFL	0.878	0.00	0.820	0.00	0.552	0.03	0.765	0.00
W-CFL	0.821	0.00	0.584	0.02	0.886	0.00	0.639	0.01

For abbreviation and other statistical detail, see caption of Table II. r, coefficient correlation; W, weight.

DISCUSSION

Probiotics use is becoming an essential part of aquaculture in gaining high quality production from aquatic animals world-wide (Nazeer *et al*., 2016). In aquaculture farming, probiotics significantly improved the growth performance and fish health (Aly *et al*., 2008; Heo *et al*., 2013). According to past literature review by author, there is no data reported on the impact of *Lactobacillus rhamnosus* GG on *Ctenopharyngodon idella*. In current study, the dietary supplementation of probiotics to fish fingerlings significantly enhanced the growth rate, feed efficiency and morphometric parameters. Highest weight gain, specific growth rate and lowest FCR was observed in D4 group. Reduced FCR represents reduction in the feed requirement for fish growth (Hardy and Kuashik, 2002). D4 group also showed improved morphometric characters and indicate positive correlation between weight and lengths of fish. The results of this experiment are similar to the outcomes of earlier studies that enhanced the growth parameters of grass carp fed with different probiotics diet (Shah *et al*., 2021; Xue *et al*., 2020). Possible reason of fish growth enhancement and feed utilization upon probiotic treatment could be due to bacterial action in establishment of normal intestinal microbiota, improving nutrition via denaturing indigestible feed components via enzymatic action and by producing vitamins including vitamin B12andbiotin (Balcázar *et al*., 2006; Planas *et al*., 2004; Suzer *et al*., 2008). The current results are supported by the conclusions of the preceding studies in tilapia (Pirarat *et al*., 2011) and *Cirrhinus molitorella* fed with *L. rhamnosus* GG probiotics diet (Yu *et al*., 2022). This strain could increase the intestinal surface area for nutrient absorption and assimilation in fish body by enhancing the height and width of villi which corresponds to increased growth rate (Caspary, 1992). *L. rhamnosus* GG cells might bound to the mucosal area of the gut wall (He *et al*., 2017). Bacterial adhesion in intestine starts the synthesis of short chain fatty acids by utilizing sugar (carbohydrates) (Cani *et al*., 2019) which triggers the release of gastrointestinal peptides or growth factors that supply energy to host organism and may confers the benefits on intestinal health (Blottiere *et al*., 2003; Pelicano *et al*., 2005) thus, enhance the feed utilization and growth of fish. *L. rhamnosus* GG administration in rats modulate the gut microbial community involved in butyrate production by the fermentation of carbohydrates, proteins and fibers (Lin *et al*., 2020). Butyrate involved in the metabolic process of bile salts and modification of toxic and mutagenic substances leading to the maintenance of intestinal health. Chickens fed with diet supplemented with *L. rhamnosus* might release some antimicrobial compounds including hydrogen peroxide, bacteriocins and organic acids having tendency to maintain beneficial intestinal flora and limit the growth of pathogens (Chen *et al*., 2016). Likewise, the dietary administration of *L. rhamnosus* GG improved the feed digestion capacity by producing the digestive enzymes or enhancing the activities of host enzymes (Wang *et al*., 2008) reported in Nile tilapia (Goncalves *et al*., 2011; Ngamkala *et al*., 2010).

CONCLUSION

Grass carp fed with commercial probiotic (PREPRO) containing *L. rhamnosus* GG at the rate of 6g per kg of basal diet showed improved growth performance, feed utilization and morphometric parameters. Hence, higher dose is suitable for rearing grass carp.

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IRB approval

The study was approved by the institutional review board of PMAS Arid Agriculture University.

Ethical statement

In our research work, we have adopted all the guidelines authorized by the ethics committee of Pir Mehr Ali Shah Arid Agricultural University Rawalpindi.

Statement of conflict of interest

The authors have declared no conflict of interests.

REFERENCES

Aly, S.M., Mohamed, M.F. and John, G., 2008. Effect of probiotics on the survival, growth and challenge infection in Tilapia nilotica (*Oreochromis niloticus*). *Aquacult. Res*., **39**: 647–656. https://doi.org/10.1111/j.1365-2109.2008.01932.x

Balcázar, J.L., Blas, I. de, Ruiz-Zarzuela, I., Cunningham, D., Vendrell, D. and Múzquiz, J.L., 2006. The role of probiotics in aquaculture. *Vet. Microbiol*., **114**: 173–186. https://doi.org/10.1016/j.vetmic.2006.01.009

Blottière, H.M., Buecher, B., Galmiche, J.P. and Cherbut, C., 2003. Molecular analysis of the effect of short-chain fatty acids on intestinal cell proliferation. *Proc. Nutr. Soc.*, **62**: 101-106. https://doi.org/10.1079/PNS2002215

Bondad-Reantaso, M.G., Subasinghe, R.P., Arthur, J.R., Ogawa, K., Chinabut, S., Adlard, R., Tan, Z. and Shariff, M., 2005. Disease and health management in Asian aquaculture. *Vet. Parasitol*., **132**: 249–272. https://doi.org/10.1016/j.vetpar.2005.07.005

Cabello, F.C., 2006. Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environ. Microbiol*., **8**: 1137–1144. https://doi.org/10.1111/j.1462-2920.2006.01054.x

Cani, P.D., Van Hul, M., Lefort, C., Depommier, C., Rastelli, M. and Everard, A., 2019. Microbial regulation of organismal energy homeostasis. *Nat. Metab*., **1**: 34–46. https://doi.org/10.1038/s42255-018-0017-4

Carnevali, O., Sun, Y.Z., Merrifield, D.L., Zhou, Z. and Picchietti, S., 2014. Probiotic applications in temperate and warm water fish species. *Aquacult. Nutr. Gut Hlth. Probiot. Prebiot*., pp. 253–289. https://doi.org/10.1002/9781118897263.ch10

Caspary, W.F., 1992. Physiology and pathophysiology of intestinal. *Am. J. clin. Nutr.,* **55**: 299S-308S. https://doi.org/10.1093/ajcn/55.1.299s
Chen, F., Gao, S.S., Zhu, L.Q., Qin, S.Y. and Qiu, H.L., 2016. Effects of dietary *Lactobacillus rhamnosus* CF supplementation on growth, meat quality, and microenvironment in specific pathogen-free chickens. *Poult. Sci*., **97**: 118–123. https://doi.org/10.3382/ps/pex261
Dimitroglou, A., Merrifield, D.L., Carnevali, O., Picchietti, S., Avella, M., Daniels, C., Güroy, D. and Davies, S.J., 2011. Microbial manipulations to improve fish health and production. A Mediterranean perspective. *Fish Shellf. Immunol*., **30**: 1–16. https://doi.org/10.1016/j.fsi.2010.08.009
El-Kady, A.A., Magouz, F.I., Mahmoud, S.A., Abdel-Rahim and M.M., 2022. The effects of some commercial probiotics as water additive on water quality, fish performance, blood biochemical parameters, expression of growth and immune-related genes, and histology of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, **546:** 737249. https://doi.org/10.1016/j.aquaculture.2021.737249
Fan, Y., Liu, L., Zhao, L., Wang, X., Wang, D., Huang, C., Zhang, J., Ji, C. and Ma, Q., 2018. Influence of *Bacillus subtilis* ANSB060 on growth, digestive enzyme and aflatoxin residue in Yellow River carp fed diets contaminated with aflatoxin B1. *Fd. Chem. Toxicol*., **113:** 108–114. https://doi.org/10.1016/j.fct.2018.01.033
FAO, 1970. *Report on the regional seminar on induced breeding of cultivated fishes,* Barrackpore, Cuttack and Bombay, India. Rome.
FAO, 2018. *The state of world fisheries and aquaculture 2018.* Meeting the sustainable development goals. Rome.
FAO, 2020. *The state of world fisheries and aquaculture 2020.* Sustainability in action. Rome.
Fuller, R., 1989. Probiotics in man and animals: A review. *J. appl. Bacteriol*., **66**: 365-378. https://doi.org/10.1111/j.1365-2672.1989.tb05105.x
Geng, X., Dong, X.H., Tan, B.P., Yang, Q.H., Chi, S.Y., Liu, H.Y. and Liu, X.Q., 2012. Effects of dietary probiotic on the growth performance, non-specific immunity and disease resistance of cobia, *Rachycentron canadum*. *Aquacult. Nutr.,* **18**: 46–55. https://doi.org/10.1111/j.1365-2095.2011.00875.x
Gonçalves, A.T., Maita, M., Futami, K., Endo, M. and Katagiri, T., 2011. Effects of a probiotic bacterial *Lactobacillus rhamnosus* dietary supplement on the crowding stress response of juvenile Nile tilapia *Oreochromis niloticus*. *Fish Sci.,* **77:** 633–642. https://doi.org/10.1007/s12562-011-0367-2
Hardy, R.W. and Kaushik S.J. 2002. Feed formulation and economic evaluation. *Fish Nutr. Acad. Proc.* Elsevier. 738.
He, S., Ran, C., Qin, C., Li, S., Zhang, H., De Vos, W.M., Ringø, E. and Zhou, Z., 2017. Anti-infective effect of adhesive probiotic lactobacillus in fish is correlated with their spatial distribution in the intestinal tissue. *Sci. Rep*., **7:** 1–12. https://doi.org/10.1038/s41598-017-13466-1
Heo, W.S., Kim, Y.R., Kim, E.Y., Bai, S.C. and Kong, I.S., 2013. Effects of dietary probiotic, *Lactococcus lactis subsp. lactis I2*, supplementation on the growth and immune response of olive flounder (*Paralichthys olivaceus*). *Aquaculture,* **346:** 20–24. https://doi.org/10.1016/j.aquaculture.2012.11.009
Kanwal, Z. and Tayyeb, A., 2019. Role of dietary probiotic ecotec in growth enhancement, thyroid tuning , hematomorphology and resistance to pathogenic challenge in *Labeo rohita* juveniles. *J. appl. Anim. Res*., **47:** 349-402. https://doi.org/10.1080/09712119.2019.1650050
Khalid, M. and Naeem, M., 2017. Morphometric relationships of length-weight and length-length of farmed *Ctenopharyngodon idella* from Muzaffar Garh, Southern Punjab, Pakistan. *Punjab Univ. J. Zool*., **32:** 57–64.
Klopper, K.B., Deane, S.M. and Dicks, L.M.T., 2018. Aciduric strains of *Lactobacillus reuteri* and *Lactobacillus rhamnosus*, isolated from human feces, have strong adhesion and aggregation properties. *Probiot. Antimicrob. Proteins*, **10:** 89–97. https://doi.org/10.1007/s12602-017-9307-5
Lara-Flores, M., Olvera-Novoa, M.A., Guzmán-Méndez, B.E. and López-Madrid, W., 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. https://doi.org/10.1016/S0044-8486(02)00277-6
Lee, W.W., Oh, J.Y., Kim, E.A., Kang, N., Kim, K.N., Ahn, G. and Jeon, Y.J., 2016. A prebiotic role of Ecklonia cava improves the mortality of Edwardsiella tarda-infected zebrafish models via regulating the growth of lactic acid bacteria and pathogen bacteria. *Fish Shellf. Immunol.*, **54**: 620–628. https://doi.org/10.1016/j.fsi.2016.05.018
Lin, R., Sun, Y., Mu, P., Zheng, T., Mu, H., Deng, F., Deng, Y. and Wen, J., 2020. *Lactobacillus rhamnosus* GG supplementation modulates the gut microbiota to promote butyrate production,

protecting against deoxynivalenol exposure in nude mice. *Biochem. Pharmacol.,* **175**: 113868. https://doi.org/10.1016/j.bcp.2020.113868

Miyauchi, E., Morita, H. and Tanabe, S., 2009. *Lactobacillus rhamnosus* alleviates intestinal barrier dysfunction in part by increasing expression of zonula occludens-1 and myosin light-chain kinase *in vivo*. *J. Dairy Sci*., **92**: 2400–2408. https://doi.org/10.3168/jds.2008-1698

Nazeer, S., Bhatti, E.M. and Begum, I., 2016. Studies on growth and immune response of *Labeo rohita* after feeding *Lactobacillus acidophillus* and *Saccharomyces cerevisiae*. *Can. J. appl. Pure Sci.,* **10**: 3991-3995.

Ngamkala, S., Futami, K., Endo, M., Maita, M. and Katagiri, T., 2010. Immunological effects of glucan and *Lactobacillus rhamnosus* GG, a probiotic bacterium, on Nile tilapia *Oreochromis niloticus* intestine with oral *Aeromonas challenges*. *Fish. Sci*., **76**: 833–840. https://doi.org/10.1007/s12562-010-0280-0

Nikoskelainen, S., Salminen, S., Bylund, G. and Ouwehand, A.C., 2001. Characterization of the properties of human- and dairy-derived probiotics for prevention of infectious diseases in fish. *Appl. environ. Microbiol*., **67**: 2430–2435. https://doi.org/10.1128/AEM.67.6.2430-2435.2001

Pandey, A., Tyagi, A. and Onkar, S., 2022. Oral feed-based administration of *Lactobacillus plantarum* enhances growth , haematological and immunological responses in *Cyprinus carpio*. *Emerg. Anim. Species*, **3**: 100003. https://doi.org/10.1016/j.eas.2022.100003

Pelicano, E., Souza, P., Souza, H., Figueiredo, M., Carvalho, S. and Bordon, V., 2005. Intestinal mucosa development in broiler. *Braz. J. Poult. Sci*., **7**: 221–229. https://doi.org/10.1590/S1516-635X2005000400005

Pirarat, N., Pinpimai, K., Endo, M., Katagiri, T., Ponpornpisit, A., Chansue, N. and Maita, M., 2011. Modulation of intestinal morphology and immunity in nile tilapia (*Oreochromis niloticus*) by *Lactobacillus rhamnosus* GG. *Res. Vet. Sci*., **91**: e92–e97. https://doi.org/10.1016/j.rvsc.2011.02.014

Pirarat, N., Pinpimai, K., Rodkhum, C., Chansue, N., Ooi, E.L., Katagiri, T. and Maita, M., 2015. Viability and morphological evaluation of alginate-encapsulated *Lactobacillus rhamnosus* GG under simulated tilapia gastrointestinal conditions and its effect on growth performance, intestinal morphology and protection against *Streptococcus agalactiae*. *Anim. Feed Sci. Technol*., **207**: 93–103. https://doi.org/10.1016/j.anifeedsci.2015.03.002

Planas, M., Vázquez, J.A., Marqués, J., Pérez-Lomba, R., González, M.P., Murado and M., 2004. Enhancement of rotifer (*Brachionus plicatilis*) growth by using terrestrial lactic acid bacteria. *Aquaculture*, **240**: 313–329. https://doi.org/10.1016/j.aquaculture.2004.07.016

Sewaka, M., Trullas, C., Chotiko, A., Rodkhum, C., Chansue, N., Boonanuntanasarn, S. and Pirarat, N., 2019. Efficacy of synbiotic *Jerusalem artichoke* and *Lactobacillus rhamnosus* GG-supplemented diets on growth performance, serum biochemical parameters, intestinal morphology, immune parameters and protection against *Aeromonas veronii* in juvenile red tilapia (*Oreochromis* spp.). *Fish Shellf. Immunol*., **86**: 260–268. https://doi.org/10.1016/j.fsi.2018.11.026

Shah, S., Chesti, A., Rather, M., Hafeez, M., Aijaz, A., Yousuf, I. and Jan, S., 2021. Effect of probiotics (*Bacillus subtilis*) on the growth and survival of fingerlings of grass Carp, *Ctenopharyngodon idella*. *Curr. J. appl. Sci. Technol*., **40**: 31–37. https://doi.org/10.9734/cjast/2021/v40i1531411

Suzer, C., Çoban, D., Kamaci, H.O., Saka, Ş., Firat, K., Otgucuoğlu, Ö. and Küçüksari, H., 2008. *Lactobacillus spp*. bacteria as probiotics in gilthead sea bream (*Sparus aurata* L.) larvae: Effects on growth performance and digestive enzyme activities. *Aquaculture*, **280**: 140–145. https://doi.org/10.1016/j.aquaculture.2008.04.020

Ullah, A., Zuberi, A., Ahmad, M., Shah, A.B., Younus, N., Ullah, S. and Khattak, M.N.K., 2018. Dietary administration of the commercially available probiotics enhanced the survival, growth, and innate immune responses in Mori (*Cirrhinus mrigala*) in a natural earthen polyculture system. *Fish Shellf. Immunol.,* **72**: 266-272. https://doi.org/10.1016/j.fsi.2017.10.056

Van Doan, H., Hoseinifar, S.H., Ringø, E., Ángeles Esteban, M., Dadar, M., Dawood, M.A. and Faggio, C., 2020. Host-associated probiotics: A key factor in sustainable aquaculture. *Rev. Fish. Sci. Aquacult.*, **1**: 16-42. https://doi.org/10.1080/23308249.2019.1643288

WHO, 2006. *Antimicrobial use in aquaculture and antimicrobial resistance*. Aeromonas Resist. How It Eff. Humans, pp. 1–107.

Wang, Y.B., 2007. Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. *Aquaculture*, **269**: 259–264. https://doi.org/10.1016/j.

aquaculture.2007.05.035

Wang, Y.B., Li, J.R. and Lin, J., 2008. Probiotics in aquaculture: Challenges and outlook. *Aquaculture*, **281**: 1–4. https://doi.org/10.1016/j.aquaculture.2008.06.002

Xue, J., Shen, K., Hu, Yi, Hu, Yajun, Kumar, V., Yang, G. and Wen, C., 2020. Effects of dietary *Bacillus cereus*, *B. subtilis*, *Paracoccus marcusii*, and *Lactobacillus plantarum* supplementation on the growth, immune response, antioxidant capacity, and intestinal health of juvenile grass carp (*Ctenopharyngodon idellus*). *Aquacult. Rep*., **17**. https://doi.org/10.1016/j.aqrep.2020.100387

Yu, Y.M., Poon, P.M.Y., Sharma, A.A., Chan, S.M.N., Lee, F.W.F., Mo, I.W.Y. and Sze, E.T.P., 2022. Colonization of *Lactobacillus rhamnosus* GG in *Cirrhinus molitorella* (Mud Carp) Fingerling: Evidence for improving disease resistance and growth performance. *Appl. Microbiol.,* **2**: 175-184. https://doi.org/10.3390/applmicrobiol2010012