Zoo Botanica
ISSN: 3007-2050 (E), 3007-2042 (P)

Check for updates

ROOTS PRESS

Research Article

Combined dietary effects of probiotics, yeast, and digestive enzymes on growth performance and meat quality in grass carp (*Ctenopharyngodon idella*)

Hajra Qamar[1], Muhammad Zubair Anjum[1], Khalid Mehmood[1], Muhammad Qayash Khan[2], Sana Urooj[1], Bushra Mushtaq[1], Tayyaba Ijaz[1], Tamom Naz[1], Vaneeza Arshad[1]

[1]*Department of Zoology, Wildlife and Fisheries, PMAS-Arid Agriculture University Rawalpindi, 46300, Pakistan.*

[2]*Department of Zoology, Faculty of Chemical and Life Sciences, Abdul Wali Khan University Mardan, KPK.*

ABSTRACT

This study investigated the effects of probiotics, yeast, and digestive enzymes as feed additive on the growth and meat quality of Grass carp. A total of 90 Fingerlings divided into three groups i.e., control (C), 2g/kg supplement (T1), and 4g/kg supplement (T2) were reared for three months in glass aquaria. The growth performance viz., weight gain, standard length, fork length and total length was measured on fortnightly basis. Proximate composition analysis was performed at the end of the experiment to evaluate meat quality of Grass carp. The group receiving 4g/kg of the supplementation (T2) shows higher values for all growth parameters, followed by T1 and C group respectively. The proximate analysis revealed significantly better meat quality, characterized by higher crude protein (64.80±1.63) with lower crude fat, moisture and ash content (16.60±0.65, 73.50±0.61, 9.13±0.41) in T2 as compared to T1 and C group. The overall outcome revealed that the supplementary diet with addition of probiotics, yeast, and digestive enzymes is a good source of nutrients and fiber for Grass carp that helps to improve the growth and meat quality of fish species.

Keywords: Grass carp; probiotics; growth performance; meat quality.

OPEN ACCESS

Correspondence
Muhammad Zubair Anjum
zubair.anjum@uaar.edu.pk

Article History
Received: July 21, 2025
Accepted: August 30, 2025
Published: December 25, 2025

INTRODUCTION

In Pakistan, the development of inland fisheries and aquaculture began in the 1970s. Apart from the marine resources freshwater fish farming is still in progress to provide protein food (Laghari, 2018). Grass carp (*Ctenopharyngodon idella*), a popular freshwater aquaculture species originally from Russia and China, is now cultivated globally. In Pakistan it was introduced from China in 1964 as a weed control. This large, slender, dark grey fish with a golden sheen belongs to the minnow family (Cyprinidae) and lacks barbels. While primarily feeding on vegetation, it also consumes animal feed. Protein and cellulose are crucial for the rapid growth of Grass carp, especially for Fingerlings (Khan et al., 2004; Cudmore et al., 2004). Grass carp, under optimal conditions, can consume large quantities of plant matter, potentially exceeding their own body weight daily. It can eat aquatic macrophytes in large amount. When food is limited or not available Grass carp can feed on any kind of plant and animal source. There are many factors that influence the feeding behavior of Grass carp like fish size, weight and age, temperature of water, presence of aquatic vegetation (Pipalova, 2006).

The use of artificial formulated feed in intensive farming has played a significant role in the ongoing increase in Grass carp production. The temporal degradation of flesh quality in aquaculture *Ctenopharyngodon idella* has elicited increasing public concern. Diet significantly influences *Ctenopharyngodon idella* growth performance and muscle quality. Supplementation with a grass-based diet can enhance flesh quality by improving muscle characteristics and collagen production, while concurrently reducing intramuscular fat and moisture content, albeit potentially at the expense of growth rate (Zhao et al., 2018). A variety of fish feed are used by farmers, such as rice polish, poultry feed, husk, gluten meal and grass. In Pakistan commercially available pelleted feed is used by far smaller percentage of farmers than in nearby nations (Rossignoli et al., 2023). Pelleted supplementary feed provides an excellent tool for managing feeding of fishes in the natural and artificial environment. Fish eat small size pelleted feed more easily and fast as compared to the larger size pelleted feed. Pelleted feed increases the fish biomass by increasing protein and fat content of fish (Akter et al., 2024).

In the field of animal nutrition, probiotics are live bacteria that are added to feed and have positive effects on the host animal. According to reports, probiotic use has led to an improvement in performance for the majority of species (Simon et al., 2001). Probiotics are mainly the microorganisms consisting bacteria, yeast and fungi. They help in the growth of fish, enhance its immunity and gut microbiota that increases digestion, ability to cope with stress and enhance reproductive ability. The filamentous fungus also lies in the category of microbes that have the probiotic attributes. The immune system and antioxidant response are boosted by fungi which also increase the synthesis of several digestive enzymes like lipases, cellulases, b-glucanases, xylanases, amylases and proteases. The other category of microbes having probiotics potential is yeast. Benefits of using yeast as probiotic in fish diets include improved immunological responses, improved growth performance, intestinal enzymatic physiology and modification of the digestive microbiota of fish (Melo et al., 2020).

In aquaculture, probiotics are gaining importance as an eco-friendly alternative to antibiotics for disease management. They offer benefits like nutrient provision, enhanced digestion (through enzymes), improved water quality, growth promotion, harmful microbe suppression, and boosted immunity in the host. Gram-positive, spore-forming Bacillus species are commonly used due to their environmental resilience and long shelf life. Specifically, *Paenibacillus polymyxa, Bacillus coagulans, Bacillus subtilis, Bacillus licheniformis,* and *Bacillus amyloliquefaciens* exhibit strong biocontrol capabilities against various fish pathogens and are administered through feed, culture water, or live feeds (Gupta et al., 2014). *Lactococcus lactis, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus sakei, Lactobacillus delbrueckii, Leuconostoc mesenteroides, Aeromonas sobria, Saccharomyces cerevisiae,* and *Carnobacterium divergens* are some of the strains of probiotics that are also used in aquaculture (Borch et al., 2015).

Feeding of common carp with diets supplemented with Synbiozyme 500 or a combination of fish-derived probiotics (*L. rhamnosus* + *L. plantarum*) for 90 days significantly improve digestive enzymes activity, immune responses and gut histology (Mohammadian et al., 2022).

Intestinal microbiota and morphology can be improved by multi-species probiotics, which can be considered an environmentally friendly growth stimulant in mrigal farming. Multiple strain probiotics increased villus area, length and width also immune response indices, including the fattening of the intestinal mucosal fold, the breadth of the lamina propria, the width of enterocytes, and the abundance of goblet cells (Hossain et al., 2022).

Over the last decade, single-strain (SSP) and multi-strain probiotics (MSP), which are microbial derivatives, have become prevalent in aquaculture. MSP are often more beneficial to aquatic organisms due to their synergistic effects compared to SSP. Most commercial probiotics claiming multiple health benefits are MSP formulations. Key advantages of MSP include preventing various freshwater and marine diseases by enhancing host survival and improving non-specific (innate and adaptive) immunity. Furthermore, hosts fed MSP frequently exhibit enhanced growth parameters, such as increased weight gain, specific growth rate, and protein efficiency ratio (Puvanasundram et al., 2021).

The aim of this study was to get a better understanding that how addition of probiotics, yeasts and digestive enzymes in supplementary feed contribute to the growth and meat quality of grass carp.

MATERIALS AND METHODS

Study site and collection of fingerlings

The experiment was conducted at Aquaculture and Fisheries laboratory, Department of Zoology, Wildlife & Fisheries, PMAS Arid Agriculture University, Rawalpindi. The Fingerlings of Grass carp were collected from the Fisheries complex, Rawal town Islamabad and was transported in polythene bags containing adequate amount of water and oxygen for safe supply to the laboratory. The commercially available feed containing probiotics (*Bacillus subtilis* and *Lactobacillus plantarum*), yeasts (*Saccharomyces cerevisiae*) and digestive enzymes (Amylase, Protease, Lipase, Pancreatin) was used to treat the experimental groups T1 (2g/kg) and T2 (4g/kg) respectively, whereas supplementary diet without addition of probiotics, yeasts and digestive enzymes was used for the control group.

Table 0. Fish feed ingredients list.

Ingredients	Crude protein level (%)
Fish meal	60-70%
Soybean meal	44-48%
Sunflower meal	30-40%
Rice polish	12-15%
Gluten 60%	60%
Wheat bran	15-18%
Vitamin premixes	-
Soybean oil	-

Supplementary feed containing essential elements keeping in mind the fish nutritional requirements.

Experimental setup

Grass carp Fingerlings were stocked and acclimatized for 3-5 days in 6 glass aquaria of equal dimensions (1x1x1.5ft/ length x width x height) equipped with aerators and filled with equal amount of water. During acclimatization period fish were fed with basal diet.

Growth experiment

The growth experiment was carried out for 90 days. 90 Fingerlings were reared in three groups (Control, Treatment T1 & Treatment T2) with (n=15/aquarium). Control group (C) was fed with commercially available supplementary feed without addition of probiotics, yeast and digestive enzymes; treatment group (T1) and treatment group (T2) were fed with commercially available supplementary feed containing 2g/kg and 4g/kg of probiotics, yeast and digestive enzymes respectively. The feed was given on daily basis @ 5% body weight of fish. The growth parameter was checked fortnightly to adjust the feed quantity for each group. To maintain the water quality, the fecal material and excessive feed was removed daily and water was exchanged in each aquaria as per need. No death was observed during experiment.

Assessment of fish growth and morphometry

The random sampling of 5 Fingerlings from each group on fortnightly basis was done to determine the growth performance of each feeding trial. Growth performance was analyzed by collecting data of fish weight and body length (Standard length, Fork length and Total length) by using standardized weighing balance, measuring board and transparent ruler. At the end of experiment, fish were deprived from feeding for one day, to determine the growth performance in terms of final body weight (g) (FBW), weight gain (g) (WG), percentage (%) weight gain (WG %). Calculations for growth performance of Grass carp were done by the following formulae described by (Panase & Mengumphan, 2015) and (Chowdhury et al., 2020).

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

Percentage weight gain (WG %) = (Average final weight (g) – Average initial weight (g) *100)/ average initial weight (g)

Morphometric parameters including Standard length (SL), Fork length (FL) and Total length (TL) were measured by standard protocol described by Apparao in 1961.

Analysis of meat quality

At the end of the feeding trail fish sample was collected to analyze the meat quality of each group through proximate composition analysis. Random sample were collected from glass aquaria and after the removal of skull, fins, scales and viscera of each sample, the meat was used for the proximate composition analysis viz., analysis of Moisture Content, Total Ash, Crude Protein, Crude Fat (AOAC, 1990). An amount of 20g fish meat sample was heated for 24 hours at 105oC in hot air oven to find out moisture content by using the formula;

$$\% \text{ moisture} = \frac{\text{Initial weight} - \text{Final weight} \times 100}{\text{Initial weight}}$$

2g of moisture free sample was placed in muffle furnace for combustion at 600^{o}C for 4 hours to calculate the ash content. Protein content was measured by using Kjeldahl technique (N×6.25). Crude fat was examined by soxhlet apparatus.

Statistical analysis

The statistical package for the social sciences (SPSS) software was used to analyze the data using analysis of variance (ANOVA) and the Duncan's multiple range test (DMRT) to identify significant differences within the treated groups. The results were presented as means± standard deviation, with a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Growth performance and feed utilization

Fish growth was observed during the 90 days' experiment on fortnightly basis. Growth performance in terms of Weight gain (WG), Standard length (SL), Fork length (FL) and Total length (TL) was measured and shown in Table1 to Table 5. Probiotics fed groups (T1 and T2) showed better growth performance and feed utilization as compared to control group. T2 showed significantly ($p<0.05$) higher weight gain (3.63±1.064g), SL (5.48±0.518cm), FL (6.08±0.526cm) and TL (6.74±0.571cm) as compared to T1 and C.

Table 1. Growth performance of grass carp fingerlings.

Weight (W)	Control	T1	T2
IBW(g)	2.40±0.219	2.42 ±0.184	2.42±0.287
FBW(g)	3.25±0.349	3.44±0.643	3.63±1.064
WG(g)	0.85±0.412	1.02±0.669	1.21±1.102
% WG	35.42%	42.15%	50.00%

Means ±SD, IBW=Initial Body Weight, FBW=Final Body Weight, WG=Weight Gain, %WG= Percentage Weight Gain.

Table 2. Fortnightly increase in the body weight (g) of Grass carp Fingerlings fed with supplementary feed containing probiotics, yeast and digestive enzymes.

Weeks	Sampling	Control (g)	T1 (g)	T2 (g)
1	1	2.40±0.219	2.42 ±0.184	2.42±0.287
2	2	2.40±0.334	2.66±0.617	2.67±0.721
3	3	2.49±0.536	2.72±0.447	2.75±0.766
4	4	2.56±0.521	2.91±0.902	2.98±1.145
5	5	2.59±0.466	3.05±0.936	3.34±0.765
6	6	3.14±0.874	3.17±0.385	3.37±0.966
7	7	3.25±0.349	3.44±0.643	3.63±1.064

T1= Treatment 1(2g/kg), T2= Treatment 2 (4g/kg); data are means ± standard deviation.

Table 3. Fortnightly increase in the standard length (cm) of grass carp fingerlings fed with supplementary feed containing probiotics, yeast and digestive enzymes.

Weeks	Sampling	Control (cm)	T1 (cm)	T2 (cm)
1	1	4.61±0.387	4.77±0.411	4.77±0.266
2	2	4.71±0.334	4.91±0.255	4.94±0.287
3	3	4.76±0.316	4.93±0.476	4.99±0.448
4	4	4.82±0.252	4.98±0.404	5.05±0.422
5	5	4.91±0.378	5.04±0.330	5.16±0.435
6	6	5.23±0.290	5.17±0.480	5.34±0.488
7	7	5.25±0.310	5.29±0.392	5.48±0.518

T1= Treatment 1(2g/kg), T2= Treatment 2 (4g/kg); data are means ± standard deviation.

Table 4. Fortnightly increase in the fork length (cm) of grass carp fingerlings fed with supplementary feed containing probiotics, yeast and digestive enzymes.

Weeks	Sampling	Control (cm)	T1 (cm)	T2 (cm)
1	1	5.26±0.437	5.29±0.347	5.29±0.251
2	2	5.34±0.236	5.42±0.339	5.53±0.442
3	3	5.35±0.287	5.57±0.290	5.54±0.323
4	4	5.45±0.435	5.57±0.421	5.56±0.414

5	5	5.46±0.429	5.75±0.424	5.80±0.503
6	6	5.79±0.237	5.90±0.487	6.03±0.533
7	7	5.90±0.266	5.96±0.427	6.08±0.526

T1= Treatment 1(2g/kg), T2= Treatment 2 (4g/kg); data are means ± standard deviation.

Table 5. Fortnightly increase in the total length (cm) of grass carp fingerlings fed with supplementary feed containing probiotics, yeast and digestive enzymes.

Weeks	Sampling	Control (cm)	T1 (cm)	T2 (cm)
1	1	5.79±0.328	5.81±0.401	5.81±0.233
2	2	6.00±0.374	6.12±0.339	6.19±0.412
3	3	5.99±0.255	6.14±0.452	6.25±0.523
4	4	6.04±0.389	6.19±0.334	6.25±0.544
5	5	6.17±0.510	6.23±0.388	6.41±0.604
6	6	6.49±0.378	6.51±0.508	6.69±0.558
7	7	6.55±0.279	6.57±0.518	6.74±0.571

T1= Treatment 1(2g/kg), T2= Treatment 2 (4g/kg); data are means ± standard deviation.

Proximate composition analysis

The experimental trial revealed significant differences in proximate composition of fish meat. The treatment 2 fed with 4g/kg of supplementary feed containing probiotics, yeasts and digestive enzymes showed highest protein content (64.80±1.63%) and lowest lipid content (16.60±0.65%), while control group showed lowest protein level (55.23±1.83%) and highest lipid level (18.35±0.50%) (Table 6). Both groups treated with probiotics, yeasts and digestive enzymes showed better meat quality as compared to control.

Table 6. Proximate composition of grass carp fed with supplementary diet containing probiotics, yeast and digestive enzymes.

Proximate Analysis	C	T1	T2
Moisture content %	80.86 ± 0.43^{a}	78.52 ± 0.93^{b}	73.50 ± 0.61^{c}
Crude protein %	55.23 ± 1.83^{c}	61.74 ± 1.89^{b}	64.80 ± 1.63^{a}
Crude fat %	18.35 ± 0.50^{a}	17.43 ± 1.00^{b}	16.60 ± 0.65^{c}
Ash %	15.66 ± 0.41^{a}	11.50 ± 0.50^{b}	9.13 ± 0.41^{c}

Data are means ± standard deviation. Superscript a, b, c, d show significant difference ($p<0.05$) across the three groups C=Control, T1=Treatment1, T2=Treatment 2.

Aquatic animals have been benefitted by the use of probiotics in terms of health, growth and survival. Probiotics can be administered orally or through water. Applications for probiotics include single or multiple strains, living or dead forms, and even in combination with immunostimulants like symbiotic, synbiotism and prebiotics. One effective way to give probiotics to aquatic animals is to encapsulate them in live feed. In order to achieve the intended benefits, dosage and time are important considerations (Hai, 2015). In current study, growth and meat quality of Grass carp was improved by the use of probiotic feed. Treatment group 2 that received 4g/kg supplementary feed showed better results as compared to T1 and Control. The administration of the probiotic feed proved to be a financially viable intervention, making animals healthier and helped them grow better. This study effectively illustrates that a modest capital outlay can catalyze significant and valuable economic returns within an aquaculture context. This study relates with previous studies carried out on probiotic feed. The use of *Bacillus subtilis* as probiotic feed showed increased Grass carp growth along with increased gut enzyme (lipase and amylase) activity (Wu et al., 2012). Aqua feed can be made from *Saccharomyces cerevisiae* biomass, which is produced as a by-product of industrial operations, and other biomasses cultivated in industry waste waters, such as *Spirulina platensis* and *Rubrivivax gelatinous*, which minimizes environmental harm and lowers disposal costs. Low levels of *S. cerevisiae*, *S. platensis*, and *R. gelatinosus* biomasses in the meal improved the nutritional value and maintained the textural characteristics of the fillets without affecting the tilapias' ability to grow (Grassi et al., 2020). Baker's yeast *Saccharomyces cerevisiae* used as supplementary feed of common carp promotes the growth of fish in terms of weight gain (Rhema & Al-Noor, 2022). The growth of grass carp was promoted by the use of multi strain probiotic when administrated with 1.68g/kg doze (Chen et al., 2020). 6g/kg of *Lactobacillus plantrum* as feed additive also increase the growth and meat quality of Grass carp (Jan et al., 2024). Similarly, the utilization of duo strain probiotic (*Bacillus subitlis* and *Lactobacillus plantrum*) through post spraying technique not only modulated digestive enzymes activity and antioxidant status but also enhanced growth of Grass carp (Luo et al., 2022).

CONCLUSION

The present study highlighted the importance of feed additives for the Grass carp. Higher concentration of supplementary feed with probiotics, yeasts and digestive enzymes resulted in improved growth performance and meat quality of Grass carp. Additionally, more research is advised to examine supplementary feed at various concentrations in order to improve outcomes for different carp species.

AUTHOR'S CONTRIBUTION

Hajra Qamar conducted experiment. Muhammad Zubair Anjum supervised the experiment. Khaild Mehmod guided throughout the experiment. Muhammad Qayash Khan provided statistical assistance. Sana Urooj, Bushra Mushtaq, Tayyaba Ijaz, TamoomNaz and Vaneeza Arshad contributed in data collection.

FUNDING

No funding was received for the present study.

AVAILABILITY OF DATA AND MATERIAL

The data supporting the findings of present research is available from the corresponding author, upon reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study did not involve human subjects or animal models. In accordance with institutional guidelines, ethical approval and informed consent requirements were formally waived for this research.

CONSENT FOR PUBLICATION

I, the undersigned, consent to the publication of my identifiable information.

CONFLICT OF INTERESTS

The authors declare there is no conflict of interest.

ACKNOWLEDGEMENT

We are grateful for the facilities provided by the Department of Zoology, Department of Zoology, Wildlife and Fisheries, PMAS-Arid Agriculture University Rawalpindi.

REFERENCES

AOAC. (1990). Official Methods of Analysis (15th ed.). K. Helrich (Ed.). Association of Official Analytical Chemists, Inc. Arlington, VA, USA.

Akter, M., Schrama, J. W., Adhikary, U., Alam, M. S., Mamun-Ur-Rashid, M., & Verdegem, M. (2024). Effect of pellet-size on fish growth, feeding behavior and natural food web in pond polyculture. Aquaculture, 593, 741342.

Apparao, B. Y. T. (1961). On some a s p e c t s of t h e biology of *lactarius lactarius* (schneider). Indian Journal of Fisheries, 13(1&2), 334–349.

Borch, K., Pederson, I. P., & Hogmo, R. O. (2015). The use of probiotics in fish feed for intensive aquaculture to promote healthy guts. International Scholars Journals, 3(7), 264–273.

Chen, X., Xie, J., Liu, Z., Yin, P., Chen, M., Liu, Y., Tian, L., &Niu, J. (2020). Modulation of growth performance, non-specific immunity, intestinal morphology, the response to hypoxia stress and resistance to *Aeromonas hydrophila* of grass carp (*Ctenopharyngodon idella*) by dietary supplementation of a multi-strain probiotic. Comparative Biochemistry and Physiology Part C Toxicology & Pharmacology, 231, 108724. https://doi.org/10.1016/j.cbpc.2020.108724

Chowdhury, M. A., Roy, N. C., & Chowdhury, A. (2020). Growth, yield and economic returns of striped catfish (*Pangasianodon hypophthalmus*) at different stocking densities under floodplain cage culture system. Egyptian Journal of Aquatic Research, 46(1), 91–95. https://doi.org/10.1016/j.ejar.2019.11.010

Cudmore, B., Mandrak, N. E., & Canada, B. O. N. L. R. (2004). Biological Synopsis of Grass Carp (*Ctenopharyngodon idella*). Canadian Manuscript Report of Fisheries and Aquatic Sciences 2705, November, 1–44. https://www.researchgate.net/publication/237457782

Gupta, A., Gupta, P., & Dhawan, A. (2014). Dietary supplementation of probiotics affects growth, immune response and disease resistance of *Cyprinus carpio* fry. Fish & Shellfish Immunology, 41(2), 113–119. https://doi.org/10.1016/j.fsi.2014.08.023

Grassi, T. L. M., Paiva, N. M., Oliveira, D. L., Taniwaki, F., Cavazzana, J. F., Da Costa Camargo, G. C. R., Diniz, J. C. P., Bermejo-Poza, R., Borghesi, R., Villarroel, M., & Ponsano, E. H. G. (2020). Growth performance and flesh quality of tilapia (*Oreochromis niloticus*) fed low concentrations of *Rubrivivax gelatinosus*, *Saccharomyces cerevisiae* and *Spirulina platensis*. Aquaculture International, 28(3), 1305–1317. https://doi.org/10.1007/s10499-020-00527-y

Hai, N. (2015). The use of probiotics in aquaculture. Journal of Applied Microbiology, 119(4), 917–935. https://doi.org/10.1111/jam.12886

Hossain, M. K., Hossain, M. M., Mim, Z. T., Khatun, H., Hossain, M. T., & Shahjahan, M. (2022). Multi-species probiotics improve growth, intestinal microbiota and morphology of Indian major carp mrigal Cirrhinuscirrhosus. Saudi Journal of Biological Sciences, 29(9), 103399.

Jan, M., Anjum, M. Z., Muhammad, Z., Farooq, M., Khan, M. Q., & Akhter, S. (2024). Effect of Commercially Available Probiotics Lactobacillus rhamnosus GG on the Body Composition of Grass Carp Fingerlings. Pakistan Journal of Zoology, 56(5). https://doi.org/10.17582/journal.pjz/20230124170136

Khan, M. S., Khan, S. A., Chaudhary, Z. I., Khan, M. N., Aslam, A., Ashraf, K., Ayyub, R. M., & Rai, M. F. (2004). Mercury intoxication in grass carp (*Ctenopharyngodon idella*). Pakistan Vet. J., 24(1), 33–38.

Laghari M Y. (2018). Aquaculture in Pakistan: Challenges and opportunities. International Journal of Fisheries and Aquatic Studies, 6(2), 56–59.

Luo, N., Wang, L., Wang, Z., Xiao, B., Wang, N., Yu, X., Wu, D., & Song, Z. (2022). Effects of dietary supplementation of duo-strain probiotics with post-spraying technology on growth performance, digestive enzyme, antioxidant capacity and intestinal microbiota of grass carp (*Ctenopharyngodon idella*). Aquaculture Reports, 26, 101301. https://doi.org/10.1016/j.aqrep.2022.101301

Mohammadian, T., Monjezi, N., Peyghan, R., & Mohammadian, B. (2022). Effects of dietary probiotic supplements on growth, digestive enzymes activity, intestinal histomorphology and innate immunity of common carp (*Cyprinus carpio*): a field study. Aquaculture, 549, 737787.

Melo-Bolívar, J. F., Ruiz-Pardo, R. Y., Hume, M. E., Sidjabat, H. E., & Villamil-Diaz, L. M. (2020). Probiotics for cultured freshwater fish. Microbiology Australia, 41(2), 105. https://doi.org/10.1071/ma20026

Pipalova, I. (2006). A Review of Grass Carp Use for Aquatic Weed Control and its Impact on Water Bodies. Journal of Aquatic Plant Management, 44, 1–12.

Panase, P., & Mengumphan, K. (2015). Growth performance, Length-Weight relationship and condition factor of backcross and reciprocal hybrid catfish reared in net cages. International Journal of Zoological Research, 11(2), 57–64. https://doi.org/10.3923/ijzr.2015.57.64

Puvanasundram, P., Chong, C. M., Sabri, S., Yusoff, M. S., & Karim, M. (2021). Multi-strain probiotics: Functions, effectiveness and formulations for aquaculture applications. Aquaculture Reports, 21, 100905. https://doi.org/10.1016/j.aqrep.2021.100905

Rossignoli, C. M., Obi, C., Ali, S. A., Ullah, N., Khalid, S., Hafeez, M., & Shah, S. M. H. (2023). Production system and challenges of saline aquaculture in Punjab and Sindh provinces of Pakistan. Frontiers in Aquaculture, 2(December). https://doi.org/10.3389/faquc.2023.1302571

Rhema, Z. A., & Al-Noor, J. M. (2022). Health and nutritional performance of young common carp *Cyprinus carpio* L. feeding diets with added bakery yeast Saccharomyces cerevisiae. International Journal of Health Sciences, 2424–2437. https://doi.org/10.53730/ijhs.v6ns6.10537

Simon, O., Jadamus, A., & Vahjen, W. (2001). Probiotic feed additives - effectiveness and expected modes of action. Journal of Animal and Feed Sciences, 10(Suppl. 1), 51–67. https://doi.org/10.22358/jafs/70012/2001

Wu, Z. X., Feng, X., Xie, L. L., Peng, X. Y., Yuan, J., & Chen, X. X. (2012). Effect of probiotic Bacillus subtilisCh9 for grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844), on growth performance, digestive enzyme activities and intestinal microflora. Journal of Applied Ichthyology, 28(5), 721–727. https://doi.org/10.1111/j.1439-0426.2012.01968.x

Zhao, H., Xia, J., Zhang, X., He, X., Li, L., Tang, R., Chi, W., & Li, D. (2018). Diet Affects Muscle Quality and Growth Traits of Grass Carp (*Ctenopharyngodon idellus*): A Comparison Between Grass and Artificial Feed. Frontiers in Physiology, 9. https://doi.org/10.3389/fphys.2018.00283