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Evaluation of Commercial Probiotic Product on Immune Function of Common Carp (*Cyprinus carpio*)

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ABSTRACT

The present study investigated the effects of graded concentrations of a commercial probiotic (PROBIO FISH) on blood parameters and non-specific immunity in common carp (*Cyprinus carpio*). Fish were fed diets supplemented with 0, 1, 2, and 2.5% commercial probiotic for a period of 56 days. A total of 120 fingerlings, with an initial mean weight of 16.75 ± 0.07g, were randomly distributed among four treatments and cultured in a closed recirculating system. Results indicated that probiotic supplementation significantly improved blood parameters and non-specific immune responses, as evidenced by increased levels of globulin, albumin, total protein, nitroblue tetrazolium, myeloperoxidase activity, serum lysozyme activity, and phagocytic activity compared with the control group. Among treatments, T2 showed significantly higher values than all other experimental groups ($P \leq 0.05$), while T1 and T3 did not differ significantly ($P \leq 0.05$) from the control. These findings suggest that dietary supplementation with the commercial probiotic PROBIO FISH can enhance immune function in common carp, supporting its potential as a functional ingredient in aquafeed formulations.

INTRODUCTION

Aquaculture is expanding worldwide, yet it continues to face significant challenges related to animal health. These challenges primarily arise from infections caused by parasites, bacteria, and viruses, which can severely impact production and profitability. Although strategies such as vaccination and antimicrobial treatments are widely applied, they have often proven insufficient for long-term disease control. Overreliance on antimicrobials not only fails to fully resolve these health issues but also contributes to environmental stress **(Torres-Maravilla *et al.*, 2024)**. The use of antibiotics to prevent disease transmission in fish farms poses considerable risks to aquaculture sustainability and human health **(Huang *et al.*, 2019; Sabah *et al.*, 2024)**.

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The emergence of antibiotic-resistant pathogens, along with growing awareness of the impacts of these chemicals on the structure of commensal microbiota in both aquatic environments and the gastrointestinal tract of fish, underscores the need for alternative, sustainable bacterial management strategies in aquaculture **(De Schryver & Vadstein, 2014)**. One such strategy is the application of probiotics—beneficial microorganisms administered to water or feed to improve water quality, immune function, and digestion. Alternative feed additives, including synbiotics, prebiotics, and probiotics, have shown promising results in enhancing aquaculture performance **(Bonadero *et al.*, 2023; Khanjani *et al.*, 2023)**. Recent studies indicate that incorporating natural sources of prebiotics, probiotics, and plant extracts into fish diets can significantly improve immune function and growth rates **(Fawole *et al.*, 2022; Huang *et al.*, 2022)**.

Probiotics such as PROBIO FISH—comprising dead or live microbial cells that are environmentally safe—promote fish health when added to feed or culture water by stabilizing gut microbiota **(Soltani *et al.*, 2019; Hussein & Jumma, 2024)**. Common probiotic strains include *Saccharomyces cerevisiae*, *Bacillus subtilis*, and *Lactobacillus* spp., often combined with growth promoters and digestive enzymes. Research has shown that PROBIO FISH supplementation improves digestion efficiency, enhances feed conversion ratios, reduces mortality rates, stimulates immune responses, and promotes microbial balance in the digestive tract **(Kuebutornye *et al.*, 2021; Soltan & El-Laithy, 2023)**.

PROBIO FISH has been tested in various economically important fish species, including common carp and the Nile tilapia. Experimental trials have demonstrated improvements in growth rates and disease resistance, particularly against bacterial and fungal pathogens associated with environmental stress or poor water quality **(Sun *et al.*, 2013; Zhou, 2022)**. Continued use of PROBIO FISH may also help reduce the accumulation of ammonia and nitrates in the aquatic environment, thereby improving water quality and overall fish health **(Farzanfar, 2006; Soltan & El-Laithy, 2023; Jumma, 2024)**.

Evaluating the performance of this probiotic under local aquaculture conditions is essential to determine its effectiveness compared with other probiotics, whether locally produced or commercially imported, as well as assessing its capacity to improve productivity and disease resistance in intensive farming systems.

MATERIALS AND METHODS

Experimental feeding of fish

The feeding experiment was conducted in the Fish Nutrition Laboratory, College of Agriculture, University of Basrah. A total of 120 common carp fingerlings were acclimatized for 14 days prior to the start of the trial. Following acclimatization, fish

were individually weighed **(**mean initial weight: 16.75 ± 0.07 g**)** and randomly assigned to 12 tanks **(**30 L capacity**)** equipped with a closed recirculating system.

Growth performance

At the end of the experimental period, fish from each tank were collected, counted, and weighed. Growth performance and efficiency metrics were calculated according to **Jobling and Koskela (1996)** as follows:

- **Weight gain (g)** = $W_2 - W_1$, where W_1 is the initial weight and W_2 is the final weight.
- **Relative growth rate (%)** = [Weight gain **(**g**)** / Initial weight **(**g**)**] × 100

Blood collection and biochemical parameters

Blood samples were collected from six fish per treatment group after 56 days. Sampling was performed via cardiac puncture using 3 mL disposable syringes, with approximately 2.5 mL of blood drawn per fish. Samples were transferred into EDTA-coated tubes and stored at –20 °C until analysis. Albumin **(**g/dL**)**, globulin, and total protein **(**g/dL**)** levels were determined using a Mindray laboratory kit and the BS-230 automated chemistry analyzer.

Non-specific immune parameters

- **Respiratory Burst Activity**: The nitroblue tetrazolium **(**NBT**)** assay was used to measure respiratory burst activity in neutrophils, based on the reduction of NBT to formazan as an indicator of superoxide anion production **(Siwicki, 1987)**.
- **Lysozyme Activity**: A turbidimetric assay was used to determine serum lysozyme activity, with hen egg white lysozyme as the standard **(Siwicki, 1987)**.
- **Phagocytic Activity**: *Micrococcus lysodeikticus* **(**0.2 mg/mL**)** was used to assess phagocytic activity **(Siwicki *et al.*, 1994)**.
- **Myeloperoxidase (MPO) Activity**: Total MPO content in serum was determined according to **Quade and Roth (1997)**.

Statistical analysis

Data were analyzed using SPSS software **(**version 22**)** to evaluate the effects of experimental treatments on measured parameters. A two-way ANOVA was applied, and mean comparisons were conducted using the Least Significant Difference **(**LSD**)** test. Statistical significance was accepted at $P \leq 0.05$.

RESULTS AND DISCUSSION

Biochemical composition of feeds

The biochemical analysis of the experimental diets showed consistent stability in feed components across all probiotic treatments **(**0.1%–0.25%**)**, with no significant differences **(**$P \leq 0.05$**)** recorded for protein, moisture, fat, carbohydrates, fiber, or ash **(**Table 1**)**. This indicates that probiotic supplementation did not alter the nutritional composition of the feed.

Growth performance

Treatment T2 **(**0.20%**)** recorded the highest growth performance **(***P*≤ 0.05**)**, with a final weight of 21.91 ± 0.06g, weight gain of 4.75 ± 0.22g, and relative growth rate of 27.67 ± 1.57%. This was followed by T3 **(**0.25%**)**, which also produced notable improvements, and T1 **(**0.10%**)**, which showed only slight enhancement over the control. The control group recorded the lowest values across all growth parameters **(**Table 2**)**.

Non-specific immunity

T2 exhibited the highest activity of non-specific immune indicators, with myeloperoxidase **(**MPO**)** at 0.20 ± 0.05% and nitroblue tetrazolium **(**NBT**)** activity at 2.07 ± 0.10%. Lysozyme activity differed significantly among treatments **(***P*≤ 0.05**)**, with T2 recording the highest value **(**32.48 ± 0.02 U/mL**)**, followed closely by T3 **(**31.51 ± 0.03 U/mL**)**. The difference between T2 and T3 was not statistically significant. Both T2 and T3 were significantly higher than T1 **(**28.03 ± 0.05 U/mL**)** and the control **(**26.95 ± 0.44 U/mL**) (**Table 3**)**.

Blood biochemical parameters

T2 demonstrated the highest levels of albumin **(**4.47 ± 0.23mg/ 100mL**)**, globulin **(**1.31 ± 0.10mg/ 100mL**)**, and total protein **(**5.53 ± 0.34 mg/100 mL**)**, with significant differences **(***P*≤ 0.05**)** compared with all other treatments **(**Table 4**)**.

Table 1. Composition of the experiment diets in the current study

Treatment	Moisture	Protein	Fat	Carbohydrates	Fiber	Ash
Control	7.2±0.24 a	27.92±0.99 a	6.10±0.11 a	44.81±0.88 a	4.91±0.30 a	9.1±0.45 a
T1 (0.1%)	7.5±0.21 a	27.92±0.05 a	6.60±2.87 a	44.91±0.71 a	4.17±0.30 b	9.0±0.15 a
T2 (0.2%)	7.6±0.24 a	27.86±0.05 a	6.30±3.12 a	44.88±0.87 a	4.16±0.29 a	9.4±0.18 a
T3 (0.25%)	7.7±0.26 a	28.00±0.06 a	6.10±3.43 a	44.10±0.92 a	4.80±0.24 a	9.5±0.16 a

The letters similar in the same row are non-significant different **(***P*≤0.05**)**.

Table 2. The feeding utilization performance and growth of *C. carp* fed diets containing different levels of commercial probiotic

Growth parameter	Control	T1 (0.1%)	T2 (0.2%)	T3 (0.25%)
Initial weight (g)	17.45±0.13 a	17.35±0.11a	17.16±0.16 b	17.31±0.02 a
Final weight (g)	18.31±0.16 d	19.35±0.08 c	21.91±0.06 a	19.98±0.29 b
Weight gain (g)	0.86±0.13 c	2.00±0.10 c	4.75±0.22 a	2.67±0.27 b
Relative growth (%)	4.94±0.79 d	11.52±0.65c	27.67±1.57 a	15.46±1.55 b

The letters different in the same row are significantly different **(***P*≤0.05**)**.

Table 3. Non-specific immunity of *C. carp* fed diets contains differently levels of commercial probiotic.

Parameters	Control	T1 (0.1%)	T2 (0.2%)	T3 (0.25%)
MPO (%)	0.146±0.03 c	0.17±0.02 b	0.20±0.05 a	0.15±0.04 b
NBT (%)	0.360±0.03 c	1.13±0.01 b	2.07±0.10 a	1.11±0.16 b
Lysozyme (units/ml)	26.9 ±0.44 c	28.03±0.05 b	32.48±0.02 a	31.51±0.03 a

The letters different in the same row are significantly differently **(***P*≤0.05**)**.

Table 4. Total protein and lipid serum profile of *C. carp* fed diets containing different levels of commercial probiotic

Parameter (mg/100 ml)	Control	T1 (0.1%)	T2 (0.2%)	T3 (0.25%)
Albumin	3.87±0.21 b	3.09±0.03 c	4.47±0.23 a	4.15±0.16 ab
Globulin	0.81±0.09 c	1.04±0.04 b	1.31±0.10 a	1.06±0.05 b
Total Protein	4.41±0.06 b	4.28±0.27 b	5.53±0.34 a	4.16±0.15 b

The letters different in the same row are significantly different **(***P*≤0.05**)**.

The results of this study clearly demonstrate the importance of supplementing diets with 0.2% imported probiotic in improving both biological performance and non-specific immunity in the common carp. The findings indicate that the probiotic's effect was achieved without altering the chemical composition of the feed, confirming that its impact can be considered an independent factor. Across all supplementation levels (0.10–0.25%), no significant differences (*P*≤ 0.05) were observed in the feed's moisture, protein, fat, carbohydrate, fiber, or ash content **(Ringø *et al.*, 2016)**. Nutritional stability in experimental diets is essential, as changes in protein or lipid content may directly influence fish immune responses, complicating interpretation **(Ringø *et al.*, 2016; Hoseinifar *et al.*, 2020; El-Sayed, 2021)**. The chemical consistency observed in the present study strengthens the validity of the biological and immune performance results by confirming that they reflect probiotic activity rather than nutrient composition changes.

Significant differences (*P*≤ 0.05) were recorded among treatments for most growth parameters, with no initial weight differences, confirming homogeneity at the start of the trial. T2 (0.2%) achieved the highest final weight, weight gain, and relative growth rate, followed by T3 (0.25%) and T1 (0.10%), while the control group consistently produced the lowest values. The superior performance of T2 can be attributed to optimal probiotic activity at this concentration, promoting digestion efficiency, nutrient absorption, and beneficial gut microbiota, which together enhance feed conversion and growth **(Merrifield *et al.*, 2009; Yao *et al.*, 2024)**.

The relative decline in growth at 0.25% (T3), despite remaining above control levels, may be linked to physiological saturation or metabolic stress caused by excessive probiotic loading. Such conditions could disrupt microbial balance in the gut or redirect energy from growth toward immune activity **(Pirarat *et al.*, 2006; Nayak, 2010)**. Conversely, the 0.10% level (T1) was likely insufficient to induce significant intestinal or absorptive changes, explaining its lower performance relative to T2. These results align with previous reports indicating that probiotic efficacy depends on achieving an optimal concentration that maximizes benefits without causing inhibitory or stressful effects **(Ganguly & Prasad, 2012; Saleh, 2024)**.

In terms of non-specific immunity, T2 again outperformed all other treatments, showing the highest lysozyme, nitroblue tetrazolium **(**NBT**)**, and myeloperoxidase **(**MPO**)**

activities. T3 and T1 also showed significant improvements over the control, though to a lesser degree. The enhanced MPO activity in T2 suggests increased immune cell capacity to produce free radicals for pathogen elimination **(Harikrishnan *et al.*, 2019; Aljoburi *et al.*, 2024)**. Similarly, higher NBT activity reflects improved phagocytic capacity to generate reactive oxygen species against microbes **(Rawling, 2012)**. Elevated lysozyme levels indicate a stronger antibacterial defense, consistent with the enzyme's key role in innate immunity **(Saurabh & Sahoo, 2008; Al-Juhaishi *et al.*, 2025)**. These observations are consistent with numerous studies reporting probiotic-mediated stimulation of innate immunity in fish, which supports disease resistance, reduces reliance on antibiotics, and promotes sustainable aquaculture **(Nayak, 2010; Standen *et al.*, 2013; Zhou, 2022; Oday *et al.*, 2024; Bashar *et al.*, 2025)**.

Blood biochemical results also support the immune-enhancing effects of T2, which recorded the highest albumin, globulin, and total protein concentrations. Increased albumin levels reflect improved protein metabolism, osmotic regulation, and biomolecule transport **(Hoseinifar *et al.*, 2018)**. Elevated globulin levels indicate greater immune activation and antibody production, consistent with probiotic-induced immunostimulation **(Standen *et al.*, 2013; Koshio & Dawood, 2016; Al-Bayati *et al.*, 2024)**. Higher total protein levels are indicative of improved nutrient absorption and general health status, as previously reported in carp and other species **(Ringø *et al.*, 2018; Rashidian *et al.*, 2020)**.

Overall, these results confirm that 0.2% is the optimal probiotic dosage for common carp under the present experimental conditions, producing superior growth, immune function, and physiological status compared with lower or higher inclusion levels. Excessive or insufficient dosages were less effective, highlighting the importance of dose optimization in probiotic-based feeding strategies for aquaculture.

CONCLUSION

The imported probiotic at a concentration of 0.2% significantly enhanced growth performance, total blood protein levels, and non-specific immune responses in common carp. It is worth noting that, it did not alter the chemical composition of the feed, confirming that these improvements were the result of its direct biological action. This concentration represents the optimal dosage, achieving a balance between enhanced biological and immune performance, without adverse effects.

REFERENCES

Ahmed, A.R.; Al-Hasson, H.A. and Al-Niaeem, K.S. (2023). The effect of Shilajit on growth performance, blood parameters, and key liver enzymes of the common carp (*Cyprinus carpio*). *Egyptian Journal of Aquatic Biology and Fisheries*, **27**(4), 683–693. https://www.ejabf.journals.ekb.eg/article_312697.html

Al-Bayati, H.H.K.; Abdullah, S.A.; Shihab, T.J.; Sultan, M. and Jumaa, Q.S. (2024). Immunogenicity of culture filtrated proteins and whole-cell killed formalin of *Listeria monocytogenes* to induced cellular immune response *in vivo*. *Open Veterinary Journal*, **14**(12), 3581–3598. https://doi.org/10.5455/OVJ.2024.v14.i12.40

Aljoburi, A.; Jumma, Q. and Al-shammari, S. (2024). Effects of different vaccination methods against Newcastle disease on immune response and some blood parameters in local chicken (*Gallus gallus domesticus*) in Shirqat city. *Assiut Veterinary Medical Journal*, **70**(183), 519–530. https://doi.org/10.21608/avmj.2024.297170.1280

Al-Juhaishi, O.A.; Abd, A.A. and Jumma, Q.S. (2025). Effects of Sulpiride on the Reproductive System of Male Rats after Puberty. *World Veterinary Journal*, **15**(1), 42–48. https://dx.doi.org/10.54203/scil.2025.wvj5

Bashar, S.N.; Qusai, S.J.; Muthanna, S.; Raghad, Z.S.; Khalid, A.H. and Ali, A.A. (2025). Preventive and ameliorative role of lactoferrin in improving blood, lipid and liver profiles toward immune homeostasis in lipopolysaccharides (LPS) induced dysregulation of bio-vital parameters in the common carp (*Cyprinus carpio*). *Egyptian Journal of Aquatic Biology and Fisheries*, **29**(2), 1173–1180. https://doi.org/10.21608/ejabf.2025.419336

Bonadero, M.C.; Del Valle, J.C. and Fernández-Gimenez, A.V. (2023). *Saccharomyces cerevisiae* as probiotic, prebiotic, synbiotic, postbiotics and parabiotics in aquaculture: An overview. *Aquaculture*, **569**, 739342. https://doi.org/10.1016/j.aquaculture.2023.739342

De Schryver, P. and Vadstein, O. (2014). Ecological theory as a foundation to control pathogenic invasion in aquaculture. *ISME Journal*, **8**, 2360–2368. https://doi.org/10.1038/ismej.2014.84

El-Sayed, A.F.M. and El-Hais, A. (2021). Probiotic use in aquafeeds: A promising strategy for aquaculture sustainability. *Aquaculture Reports*, **20**, 100722. https://doi.org/10.3390/ani14243644

Farzanfar, A. (2006). The use of probiotics in shrimp aquaculture. *FEMS Immunology & Medical Microbiology*, **48**(2), 149–158. https://doi.org/10.1111/j.1574-695X.2006.00116.x

Fawole, F.J.; Yisa, R.O.; Jayeoba, O.O.; Adeshina, I.; Ahmed, A.O. and Emikpe, B.O. (2022). Effect of dietary polyherbal mixture on growth performance, haemato-immunological indices, antioxidant responses, and intestinal morphometry of African catfish, *Clarias gariepinus*. *Aquaculture Nutrition*, **2022**, 1–11. https://doi.org/10.1155/2022/5502796

Ganguly, B. and Prasad, S. (2012). Homology modeling and functional annotation of bubaline pregnancy associated glycoprotein 2. *Journal of Animal Science and Biotechnology*, **3**(1), 13. https://doi.org/10.1186/2049-1891-3-13

Global Market Insights Inc. (2022). *Animal Feed Probiotics Market Size by Livestock, Industry Analysis Report, Regional Outlook, Application Growth Potential, Competitive Market Share & Forecast, 2022–2030*. Retrieved from https://www.gminsights.com/industry-analysis/animal-feed-probiotics-market

Harikrishnan, R.; Kim, M.C. and Balasundaram, C. (2019). Immunomodulatory effect of probiotics in aquaculture. *Fish & Shellfish Immunology*, **83**, 334–345.

Hoseinifar, S.H.; Sun, Y.Z.; Wang, A. and Zhou, Z. (2018). Probiotics as means of diseases control in aquaculture, a review of current knowledge and future perspectives. *Frontiers in Microbiology*, **9**, 2429. https://doi.org/10.3389/fmicb.2018.02429

Hoseinifar, S.H.; Van Doan, H.; Faggio, C.; Chitmanat, C. and Ringø, E. (2020). The effect of dietary probiotics on the growth and health of fish – a review. *Aquaculture Research*, **51**(1), 1–12. https://doi.org/10.1111/are.14376

Huang, C.; Yang, X.; Huang, J.; Liu, X.; Yang, X.; Jin, H.; Huang, Q.; Li, L. and Zhou, R. (2019). Porcine Beta-Defensin 2 provides protection against bacterial infection by a direct bactericidal activity and alleviates inflammation via interference with the TLR4/NF-κB pathway. *Frontiers in Immunology*, **10**, 1673. https://doi.org/10.3389/fimmu.2019.01673

Huang, X.; Li, W.; Lu, X.; Liu, C.; Huang, Y. and Su, Y. (2022). Effects of dietary *Lactobacillus reuteri* on growth performance, nutrient retention, gut health and microbiota of the Nile tilapia (*Oreochromis niloticus*). *Aquaculture Reports*, **26**, 101275. https://doi.org/10.1016/j.aqrep.2022.101275

Hussein, A. and Jumma, Q. (2024). Diagnosis of infectious bronchitis infection in broiler chicken farms in Salah Al-Din Governorate. *Egyptian Journal of Veterinary Sciences*, **55**(6), 1619–1626. https://doi.org/10.21608/ejvs.2024.262773.1780

Jobling, M. and Koskela, J. (1996). Interindividual variations in feeding and growth in rainbow trout during restricted feeding. *Aquaculture*, **145**(3–4), 245–253. https://doi.org/10.1016/S0044-8486(96)01344-8

Jumma, Q. (2024). Detection of ESBL *E. coli* that carried STX1 and STX2 form common carp (*Cyprinus carpio*) in Salhaldeen Province. *Egyptian Journal of Veterinary Sciences*, **55**(4), 1165–1170. https://doi.org/10.21608/ejvs.2024.255648.1728

Khalil, H.S.; El-Dakar, A.Y. and Shalaby, A.M. (2022). Dietary *Lactobacillus acidophilus*-enhanced growth, immune responses, antioxidant capacity, and resistance of common carp (*Cyprinus carpio*) against *Aeromonas hydrophila*. *Aquaculture Reports*, **25**, 100867. https://doi.org/10.1016/j.aqrep.2022.100867

Khanjani, M.H.; da Silva, L.O.B.; Fóes, G.K.; do Nascimento Vieira, F.; Poli, M.A.; Santos, M. and Emerenciano, M.G.C. (2023). Synbiotics and aquamimicry as alternative microbial-based approaches in intensive shrimp farming and biofloc: Novel disruptive techniques or complementary management tools? A scientific-based overview. *Aquaculture*, **567**, 739273. https://doi.org/10.1016/j.aquaculture.2023.739273

Koshio, S. and Dawood, M.A.O. (2016). Recent advances in the role of probiotics and prebiotics in carp aquaculture: A review. *Aquaculture*, **454**, 243–251. https://doi.org/10.5555/20163052482

Kuebutornye, F.K.A.; Abarike, E.D. and Lu, Y. (2020). A review on the application of *Bacillus* as probiotics in aquaculture. *Fish & Shellfish Immunology*, **87**, 820–828. https://doi.org/10.1016/j.fsi.2019.02.010

Merrifield, D.L.; Dimitroglou, A.; Bradley, G.; Baker, R.T. and Davies, S.J. (2009). Soybean meal alters autochthonous microbial populations, microvilli morphology and compromises intestinal enterocyte integrity of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases*, **32**(9), 755–766. https://doi.org/10.1111/j.1365-2761.2009.01052.x

Mohammadi, M.; Mirzargar, S.S. and Edalat, R. (2022). Effects of dietary supplementation with *Lactobacillus delbrueckii* on growth performance, immune parameters, and disease resistance of rainbow trout (*Oncorhynchus mykiss*). *Probiotics and Antimicrobial Proteins*, **14**(3), 509–517. https://doi.org/10.1007/s12602-021-09825-6

Nayak, S.K. (2010). Probiotics and immunity: a fish perspective. *Fish & Shellfish Immunology*, **29**(1), 2–14. https://doi.org/10.1016/j.fsi.2010.02.017

NRC (National Research Council). (2011). *Nutrient Requirements of Fish and Shrimp*. National Academies Press, Washington, D.C. https://nap.nationalacademies.org/catalog/13039/nutrient-requirements-of-fish-and-shrimp

Oday, A.A.; Firas, A.H.; Qusai, S.J. and Marwa, A.H. (2024). Histological investigation of the skin structure in the common carp (*Cyprinus carpio*) and the catfish (*Silurus triostegus*): Aquatic environment adjustment. *Egyptian Journal of Aquatic Biology and Fisheries*, **28**(6), 2219–2228. https://doi.org/10.21608/ejabf.2024.400587

Pirarat, N.; Kobayashi, T.; Katagiri, T.; Maita, M. and Endo, M. (2006). Protective effects and mechanisms of a probiotic bacterium *Lactobacillus rhamnosus* against experimental *Edwardsiella tarda* infection in tilapia (*Oreochromis niloticus*). *Veterinary Immunology and Immunopathology*, **113**(3–4), 339–347. https://doi.org/10.1016/j.vetimm.2006.06.003

Quade, M.J. and Roth, J.A. (1997). A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. *Veterinary Immunology and Immunopathology*, **58**(3–4), 239–248. https://doi.org/10.1016/S0165-2427(97)00048-2

Rashidian, G.; Nematollahi, A. and Shokrpoor, S. (2020). Effects of a commercial probiotic (Probio-Fish) on growth performance, immune response and disease resistance in common carp (*Cyprinus carpio*). *Aquaculture Reports*, **17**, 100319.

Rawling, M.D. (2012). Non-specific immune response of fish to probiotics: A review. *Aquaculture Research*, **43**(6), 857–866.

Ringø, E.; Hoseinifar, S.H.; Ghosh, K.; Doan, H.V.; Beck, B.R. and Song, S.K. (2018). Lactic acid bacteria in finfish – an update. *Frontiers in Microbiology*, **9**, 1818. https://doi.org/10.3389/fmicb.2018.01818

Ringø, E.; Hoseinifar, S.H.; Ghosh, K.; Doan, H.V.; Beck, B.R. and Song, S.K. (2016). Lactic acid bacteria in finfish – an update. *Frontiers in Microbiology*, **7**, 153. https://doi.org/10.3389/fmicb.2016.00153

Sabah, M.A.; Qusai, J. and Ali, A. (2024). Oxidative stress and blood parameters affected by carbaryl insecticide in *Cyprinus carpio*. *Egyptian Journal of Aquatic Biology and Fisheries*, **28**(6), 2195–2201. https://doi.org/10.21608/ejabf.2024.400585

Sabo da Silva, S.; Figueroa Villalobos, E.; Meireles Piazentin, A.C.; Lopes, A.M. and de Souza Oliveira, R.P. (2020). Impact of probiotics on animal health. In *A.M. Holban & A.M. Grumezescu (Eds.), Probiotics in The Prevention and Management of Human Diseases* (pp. 333–350). Academic Press. https://doi.org/10.1201/9780429422591-15

Safari, R.; Irani, N.; Marammazi, J.G. and Poursaeid, S. (2023). Effects of multispecies probiotic combination on growth performance, intestinal morphology, and expression of immune-related genes in common carp (*Cyprinus carpio* L.). *Aquaculture International*, **32**, Article 20. https://doi.org/10.1007/s10499-025-02035-3

Saleh, Q.J. (2024). Detection of ESBL *E. coli* that carried STX1 and STX2 form common carp (*Cyprinus carpio*) in Salhaldeen Province. *Egyptian Journal of Veterinary Sciences*, **55**(4), 1165–1170. https://doi.org/10.21608/ejvs.2024.255648.1728

Saurabh, S. and Sahoo, P.K. (2008). Lysozyme: an important defense molecule of fish innate immune system. *Aquaculture Research*, **39**(3), 223–239. https://doi.org/10.1111/j.1365-2109.2007.01883.x

Singhrong, W.; Yunchalard, S. and Sangsopha, W. (2023). Probiotics and their impact on intestinal health and immunity in aquaculture species. *Aquaculture Reports*, **28**, 101469. https://doi.org/10.1016/j.aqrep.2023.101469

Siwicki, A. (1987). Immunomodulating activity of levamisole in carp spawners, *Cyprinus carpio* L. *Journal of Fish Biology*, **31**(sA), 245–246. https://doi.org/10.1111/j.1095-8649.1987.tb05325.x

Soltan, M.A. and El-Laithy, S.M. (2023). Application of probiotics in aquaculture: Effects on growth performance and immunity in fish. *Aquaculture International*, **31**, 503–518. https://doi.org/10.1007/s10499-023-00990-9

Soltani, M.; Ghosh, K.; Hoseinifar, S.H.; Kumar, V.; Lymbery, A.J.; Roy, S. and Ringø, E. (2019). Genus *Bacillus*, promising probiotics in aquaculture: aquatic animal origin, bio-active components, bioremediation and efficacy in fish and shellfish. *Reviews in Fisheries Science and Aquaculture*, **27**(3), 331–379. https://doi.org/10.1080/23308249.2019.1597010

Standen, B.T.; Rawling, M.D.; Davies, S.J.; Castex, M.; Foey, A.; Gioacchini, G. et al. (2013). Probiotic *Pediococcus acidilactici* modulates both localised intestinal- and peripheral-immunity in tilapia (*Oreochromis niloticus*). *Fish & Shellfish Immunology*, **35**(4), 1097–1104. https://doi.org/10.1016/j.fsi.2013.07.018

Sun, Y.Z.; Yang, H.L.; Huang, K.P.; Ye, J.D. and Zhang, C.X. (2013). Application of autochthonous *Bacillus* bioencapsulated in copepod to grouper *Epinephelus coioides* larvae. *Aquaculture*, **392–395**, 44–50. https://doi.org/10.1016/j.aquaculture.2013.01.037

Torres-Maravilla, E.; Parra, M.; Maisey, K.; Vargas, R.A.; Cabezas-Cruz, A.; Gonzalez, A. et al. (2024). Importance of probiotics in fish aquaculture: Towards the identification and design of novel probiotics. *Microorganisms*, **12**(3), 626. https://doi.org/10.3390/microorganisms12030626

Wertz, A.E.; El-Sayed, A.F.M. and Ringo, E. (2023). Application of probiotics as alternatives to antibiotics in animal and fish feeds: Current trends and future perspectives. *Aquaculture Nutrition*, **29**(2), 765–777. https://doi.org/10.1002/aqn.10345

Yao, Y.; Wang, X.; Lin, X.; Wu, J.; Wang, P.; Zhu, C. and Yan, Q. (2024). Isolation and characterization of probiotic *Lysinibacillus* species from the gastrointestinal tract of large yellow croaker (*Larimichthys crocea*). *Fish & Shellfish Immunology*, **166**, 110610. https://doi.org/10.1016/j.fsi.2025.110610

Zhou, X.; Wang, Y. and Li, W. (2020). Effects of probiotic supplementation on the growth and health of juvenile fish. *Reviews in Aquaculture*, **12**(1), 60–75.

Zhou, Y. (2022). Probiotics enhance growth performance and immune response in aquaculture species: A review. *Frontiers in Microbiology*, **13**. 845789.