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Probiotics on Commercial Fish Growth: A Meta-Analysis

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Probiotics are widely used in fish diets to improve health and growth, but a detailed analysis of their impact on fish growth performance has been lacking. This study conducted a meta-analysis of 86 relevant articles out of 627 identified, focusing on specific growth rate (SGR) and feed conversion ratio (FCR). The study examined the effects of different variables, including the number of probiotic strains used, the type of water (freshwater, saltwater, or brackish), and the concentration of probiotics. The results indicated that probiotics had a significant positive effect on both SGR and FCR in fish. There was no notable difference in growth performance between diets with single strains versus multiple strains of probiotics. However, freshwater fish showed a better response to probiotics compared to saltwater and brackish water fish. The analysis found that the minimum effective concentration of probiotics for improving SGR was 7 log CFU/g, while 8 log CFU/g was needed to enhance FCR. Overall, this meta-analysis offers valuable insights into optimizing the use of probiotics in aquaculture, demonstrating that specific factors such as water type and probiotic concentration play critical roles in achieving the best growth performance in fish.

Key words: FCR, fish feed, probiotic, SGR.

INTRODUCTION

Aquaculture, the farming of fish and other aquatic organisms play a crucial role in meeting the increasing global demand for seafood. Optimizing fish growth and health is of paramount importance to ensure sustainable and efficient production. In recent years, probiotics have emerged as a promising and eco-friendly approach for enhancing fish growth and overall performance in commercial aquaculture settings (Chauhan and Singh 2019). Probiotics also play a role in mitigating disease risks and improving overall fish health by supporting the natural defence mechanisms of fish. Probiotics are live microorganisms that confer health benefits to hosts when administered in adequate amounts. In commercial fish farming, probiotics are added to fish diets or directly to aquaculture environments. They can positively influence various aspects of fish physiology, metabolism, and immunity, improve growth rates, improve feed utilization, and enhance disease resistance (Nayak 2010). The potential of probiotics to enhance the growth of commercial fish has sparked interest among researchers and aquaculture practitioners worldwide. Studies have been conducted on a wide range of commercially important fish species, such as salmon (Cather *et al.* 2022), tilapia (Apún-Molina *et al.* 2017), catfish (Manopo *et al.* 2019), and shrimp (Wei *et al.* 2022) to assess the effects of different probiotic strains and formulations.

Research on fish growth not only focuses on growth performance but also on other critical aspects of fish health and aquaculture sustainability. Probiotics have been shown to positively modulate the fish gut microbiota, improve nutrient absorption, and reduce the need for antibiotic treatments, this contributes to a healthier and more sustainable aquaculture system. The presence of beneficial probiotic bacteria in the gut can enhance the digestion and absorption of nutrients, leading to more efficient growth and reduced feed wastage (Meidong *et al.* 2017; Nathanailides *et al.* 2021; Ghori *et al.* 2022). Although the benefits of probiotics on commercial fish growth are promising, challenges remain in identifying the most effective probiotic strains, determining optimal dosage levels, and understanding the mechanisms behind their positive effects.

This introduction sets the stage for a comprehensive exploration of the current state of probiotics in commercial fish growth. By delving into the latest research, practical applications, and future perspectives, we aimed to provide valuable insights for aquaculture professionals, researchers, and policymakers. Together, we can harness the potential of probiotics to foster sustainable and efficient aquaculture practices, ensuring a bountiful supply of healthy seafood for generations (Merrifield *et al.* 2010). Understanding the probiotic effect on fish growth is essential for sustainable aquaculture development and for meeting the ever-growing

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demand for high-quality seafood. This exploration provides valuable insights for fish farmers, researchers, and stakeholders in the aquaculture industry. Together, we can unlock the full potential of probiotics to revolutionize fish farming, foster healthier fish populations, and contribute to a more sustainable aquaculture future (Subasinghe *et al.* 2009).

In the dynamic world of commercial aquaculture, maximizing fish growth and optimizing feed efficiency are critical for sustainable and profitable fish farming operations. As a growing body of research has explored the potential benefits of probiotics in aquaculture, attention has turned to two vital performance indicators: the Specific Growth Rate (SGR) and the Feed Conversion Ratio (FCR). Specific Growth Rate (SGR) is a key metric for assessing the growth performance of fish in aquaculture systems. It quantifies the rate at which fish increases in size over a specific period, typically expressed as a percentage increase in body weight per day. A higher SGR indicates faster growth, which is a desirable outcome for commercial fish farmers aiming to reduce production time and increase yield (Md. Hashibur *et al.* 2022). The Feed Conversion Ratio (FCR) is another crucial parameter that measures feed efficiency in aquaculture. It was calculated by dividing the total feed given to the fish by the total weight gain during the same period. A lower FCR indicates more efficient conversion of feed into fish biomass, signifying that the fish utilize the provided feed effectively for growth (Besson *et al.* 2020). The study examined the effects of different variables, including the number of probiotic strains used, the type of water (freshwater, saltwater, or brackish), and the concentration of probiotics.

MATERIALS AND METHODS

The study utilized several materials to conduct the meta-analysis, including the meta-analysis tool OpenMee, Microsoft Excel, reference management software such as Mendeley and Zotero, and online search engines. The study involved distinct stages, namely identification, selection, and suitability assessment of relevant articles, with the final inclusion of appropriate articles for the meta-analysis.

Literature Search and Selection Method. The literature search was performed using reputable scientific search engines, such as ScienceDirect (www.sciencedirect.com) and PubMed (pubmed.ncbi.nlm.nih.gov). The search terms used were "Probiotic," "Fish," and "Growth." The initial search yielded a total of 627 potential articles. The selection process involved specific criteria: (1) publication in English, (2) publication in peer-reviewed journals, (3) utilization of probiotics as a primary component in fish diets, and (4) availability of essential data such as mean, standard deviation/standard error, and sample size. After screening, 86 articles met the inclusion criteria and were included in the meta-analysis. Detailed information regarding the selection process can be found in the PRISMA-P flowchart (1).

Data Analysis. The data analysis involved the utilization of the OpenMee software to calculate the effect size and standard error of the effect size.

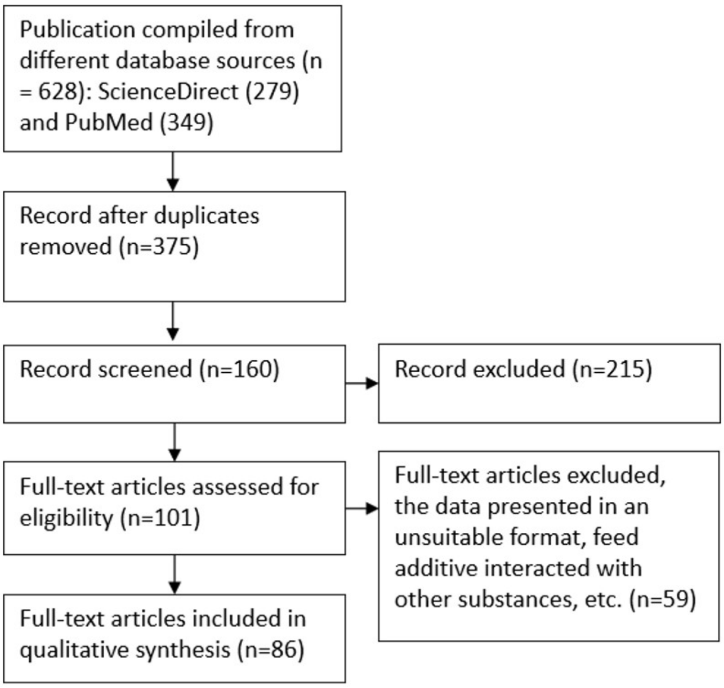


Figure 1. Flow chart of selection processes acording to PRISMA protocols

Additionally, the Rosenthal fail-safe number was used to assess the publication bias. The effect size was determined using Hedges'd method, which was chosen for its ability to quantify the effect size under conditions of heterogeneity (Sánchez-Meca and Marín-Martínez 2010). In the analysis, the probiotic-supplemented group was combined into the experimental group (E), whereas the group without probiotic addition was consolidated as the control group (C). The effect size (d) was calculated using the following formula Eq (1):

$$d = \frac{(\bar{X}^E - \bar{X}^C)}{S} \times J \qquad (1)$$

is the mean of the experimental group and is the mean value of the control group. J is the correction factor for the small sample size Eq (2):

$$J = 1 - \frac{3}{(4\,(N^C + N^E - 2) - 1)} \qquad (2)$$

Where S is the standard deviation Eq (3):

$$S = \sqrt{\frac{(N^E - 1)\,(s^E)^2 + (N^C - 1)\,(s^C)^2}{(N^E + N^C - 2)}} \qquad (3)$$

N^E is the sample size of experimental group, N^C is the sample size of the control group, s^E is the standard deviation of the experimental group, and s^C is the standard deviation of the control group. Hedges' d variance (Vd) is described as follows Eq (4):

$$V_d = \frac{(N^C - N^E)}{N^C\,N^E} + \frac{d^2}{2(N^C + N^E)} \qquad (4)$$

The cumulative effect size (d++) was calculated as follows Eq (5):

$$d_{++} = \frac{(\Sigma_{i=1}^{n}\, w i d i)}{(\Sigma_{i=1}^{n}\, w i)} \qquad (5)$$

w*i* is the inverse of the sampling variance,

Effect size precision is described using a 95% confidence interval (CI). All the above equations were derived by Sanchez-Meca and Marin-Martinez (Sánchez-Meca and Marín-Martínez 2010). The effect size is statistically significant when CI does not reach the null effect size.

The publication bias test on this meta-analysis was using Rosenthal fail-safe numbers. Rosenthal fail-safe numbers have been used for identifying the publication bias in this meta-analysis. The fail-safe number (N_{fs}) was calculated to recognize the publication bias caused by the insignificant article which is not included in the analysis. is the alternative hypothesis that was used to provide the publication bias on this meta-analysis (Fragkos *et al.* 2014).

RESULTS

Research on the application of probiotics in fish has been conducted in various countries. In this meta-analysis, the majority of articles were derived from countries in Asia and Africa, with China, Iran, and Thailand being the most represented (Table 1). From the diverse studies conducted, it is evident that Oreochromis niloticus is the most commonly utilized fish species for probiotic application, as indicated by its distribution in articles from Asia, Africa, and Europe (Table 1). Among the probiotic groups investigated in this research, the lactic acid bacteria (LAB) group, including *Lactobacillus, Lactococcus, Enterococcus, Streptococcus* etc., was predominantly employed, along with non-LAB bacteria such as *Bacillus*, and a smaller representation from the yeast group, *Saccharomyces cerevisiae* (Table 1).

The growth parameters analyzed in this meta-analysis were specific growth rate (SGR) and feed conversion ratio (FCR). The relationship between Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) is pivotal in understanding fish growth in aquaculture. Typically, a higher SGR indicates that fish are growing efficiently, which often correlates with a lower FCR, meaning they require less feed to achieve weight gain. This inverse relationship suggests that when fish convert feed into body mass effectively, their growth rates improve. Factors such as diet quality, environmental conditions, and species-specific traits can significantly influence both SGR and FCR. By monitoring these metrics, aquaculturists can optimize feeding strategies and environmental management to enhance growth rates and feed efficiency, ultimately leading to more sustainable and profitable fish production. The comparison of 195 studies on SGR showed significant results regarding the use of probiotics in fish feed (Table 2). The cumulative effect size obtained for SGR was positive value (based on the growth of fish over a specific period), indicating that probiotic supplementation in fish feed can enhance the overall SGR of fish (p-value<0.001). The increase in SGR in the aquaculture industry is an important factor, as it provides information on the growth rate of fish in response to treatment. Similarly, FCR was significantly influenced by probiotic supplementation. Out of the 182 studies compared, the cumulative effect size for FCR was negative, suggesting that probiotics in fish feed can significantly reduce FCR values (p-value<0.001). The reduction in FCR is important in the aquaculture industry because it improves the efficiency of feed utilization for fish growth.

Table 1. Studies selected for meta-analysis

Study	Country	Fish species	Probiotics
Tabassum *et al.* 2021	Bangladesh	*Oreochromis niloticus*	*Bacillus bacteria, Streptococcus faecalis, Clostridium butyricum*
Islam *et al.* 2021	Bangladesh	*Oreochromis niloticus*	*Saccharomyces cerevisiae*
Paixãoa *et al.* 2020	Brazil	*Amphiprion ocellaris*	*Lactobacillus plantarum*
Suphoronski *et al.* 2021	Brazil	*Oreochromis niloticus*	*Enterococcus faecium*
Tachibana *et al.* 2020	Brazil	*Oreochromis niloticus*	*Enterococcus faecium*
Gong *et al.* 2019	China	*Ctenopharyngodon idella*	*Pediococcus pentosaceus* SL001
Liu *et al.* 2017	China	*Oreochromis niloticus*	*Bacilus subtilis* HAINUP40
Wang *et al.* 2021a	China	*Cynoglossus semilaevis*	*Bacillus subtilis*
Zhang *et al.* 2022a	China	*Siniperca chuatsi*	*Clostridium butyricum, Enterococcus faecalis, Bacilus subtilis, Lactobacillus plantarum*
Yin *et al.* 2021	China	*Larimichthys crocea*	*Clostridium butyricum*
Abarike *et al.* 2018	China	*Oreochromis niloticus*	*Bacillus subtilis, Bacillus licheniformis*
Fei *et al.* 2018	China	*Acipenser schrenkii* x *Acipenser baeri*	*Bacillus amyloliquefaciens* (GB-9)
Qin *et al.* 2020	China	*Ctenopharyngodon idella*	*Bacillus licheniformis*
Xue *et al.* 2020	China	*Ctenopharyngodon idella*	*Bacillus cereus, Bacillus subtilis, Paracoccus marcusii, Lactobacillus plantarum*
He *et al.* 2013	China	*Oreochromis niloticus* x *Oreochromis aureus*	*Bacillus subtilis* C-3102
Zhu *et al.* 2021	China	*Siniperca chuatsi*	*Lactococcus lactis* 3-c-18
Li *et al.* 2020	China	*Paralichthys olivaceus*	*Lactobacillus plantarum*
Song *et al.* 2006	China	*Miichthys miiuy*	*Clostridium butyricum*
Cao *et al.* 2019	China	*Carassius auratus* var. *Pengze*	*Bacillus subtilis*
Yu *et al.* 2018	China	*Carassius auratus gibelio*	*Bacillus coagulans*
Wang *et al.* 2019	China	*Salmo salar* L.	*Bacillus velezensis* V4, *Rhodotula mucilaginosa*
Kuebutornye *et al.* 2020	China	*Oreochromis niloticus*	*Bacillus velezensis* TPS3N, *Bacillus subtilis* TPS4, *Bacillus amyloliquefaciens* TPS17
Yang *et al.* 2020	China	*Carassius auratus* var. *Pengze*	*Bacillus cereus*
Wang *et al.* 2021b	China	*Siniperca chuatsi*	*Bacillus velezensis* GY65
Li *et al.* 2019	China	*Oreochromis niloticus*	*Clostridium butyricum*
Feng *et al.* 2022	China	*Cyprinus carpio*	*Lactococcus lactis* Q-8, *Lactococcus lactis* Q-9, *Lactococcus lactis* Z-2
Magouz *et al.* 2022	Egypt	*Liza ramada*	*Bacillus subtilis*
Khalafalla *et al.* 2022	Egypt	*Liza ramada*	*Lactobacillus acidophilus* ATCC 4356
Hamdan *et al.* 2016	Egypt	*Oreochromis niloticus*	*Lactobacillus plantarum* AH 78
Makled *et al.* 2020	Egypt	*Oreochromis niloticus*	*Psychrobacter maritimus* S
Gayed *et al.* 2021	Egypt	*Oreochromis niloticus*	*Ruminococcus flavefaciens*
Kord *et al.* 2021	Egypt	*Oreochromis niloticus*	*Saccharomyces cerevisiae, Lactobacillus* sp., *Bacillus subtilis, Bacillus licheniformis, Bacillus pumilus*
Al-Deriny *et al.* 2020	Egypt	*Oreochromis niloticus*	*Bacillus amyloliquefaciens*
Fuchs *et al.* 2015	Germany	*Scopthalmus maximus*	*Bacillus subtilis, Bacillus licheniformis*
Midhun *et al.* 2019a	India	*Oreochromis niloticus*	*Paenibacillus polymyxa* HGA4C
Bhatnagar and Lamba 2015	India	*Cirrhinus mrigala*	*Bacillus cereus*
Jinendiran *et al.* 2021	India	*Cyprinus carpio*	*Aeromonas veronii* V03
Midhun *et al.* 2019b	India	*Oreochromis niloticus*	*Bacillus licheniformis* HGA8B
Singh *et al.* 2019	India	*Labeo rohita*	*Bacillus circulans*
Mani *et al.* 2021	India	*Cyprinus carpio*	*Lysinibacillus macroides*
Das *et al.* 2021	India	*Heteropneustes fossilis*	*Streptomyces antibioticus, Bacillus cereus*
Widanarni and Tanbiyaskur 2015	Indonesia	*Oreochromis niloticus*	*Bacillus* sp. NP5
Utami *et al.* 2015	Indonesia	*Oreochromis niloticus*	*Bacillus* NP5
Farsani *et al.* 2020	Iran	*Oncorhynchus mykiss*	*Lactobacillus acidophilus, L. casei, Enterococcus faecium, Bifidobacterium bifidum*
Mehdinejad *et al.* 2018	Iran	*Carassius auratus*	*Pediococcus acidilactici*
Tarkhani *et al.* 2020a	Iran	*Rutilus rutilus caspicus*	*Pediococcus acidilactici, Enterococcus faecium* CGMCC1.2136
Mirghaed *et al.* 2018	Iran	*Rutilus frisii kutum*	*Lactobacillus acidophilus, L. casei, Enterococcus faecium, Bifidobacterium bifidum*
Soltani *et al.* 2019	Iran	*Oncorhynchus mykiss*	*Lactobacillus plantarum*

Table 1. Continued

Study	Country	Fish species	Probiotics
Firouzbakhsh *et al.* 2011	Iran	*Astronotus ocellatus*	*Lactobacillus plantarum, L. delbrueckii. L. acidophilus, L. rhamnosus, Bifidobacterium bifidum, Streptococcus silivarius, Enterococcus faecium, Aspergillus oryzae, Candida pintolopesii*
Mohammadi *et al.* 2020	Iran	*Oreochromis niloticus*	*Lactobacillus plantarum* (KC426951)
Hooshyar *et al.* 2020	Iran	*Oncorhynchus mykiss*	*Lactobacillus rhamnosus* ATCC 7469
Akbari *et al.* 2021	Iran	*Cyprinus carpio*	*Enterococcus casseliflavus* EC-001
Mohammadi *et al.* 2015	Iran	*Amatitlania nigrofasciata*	*Saccharomyces cerevisiae*
Yeganeh *et al.* 2021	Iran	*Oncorhynchus mykiss*	*Lactococcus lactis* subsp. *lactis* PTCC 1403
Tarkhani *et al.* 2020b	Iran	*Rutilus rutilus caspicus*	*Enterococcus faecium* CGMCC1.2136
Sahandi *et al.* 2019	Iran	*Oncorhynchus mykiss*	*Bifidobacterium lactis* PTCC-1631, *Bifidobacterium lactis* PTCC-1737
Giorgia *et al.* 2018	Italy	*Oreochromis niloticus*	*Lactobacillus rhamnosus* IMC501
Shadrack *et al.* 2021	Japan	*Seriola dumerili*	*Bacillus amyloliquefaciensm, Streptococcus faecalis, Lactobacillus plantarum, Bacillus mesentericus*
Opiyo *et al.* 2019	Kenya	*Oreochromis niloticus*	*Saccharomyces cerevisiae, Bacillus subtilis*
Munir *et al.* 2016	Malaysia	*Channa striata*	*Saccharomyces cerevisiae*
Talpur *et al.* 2014	Malaysia	*Channa striata*	*Lactobacillus acidophilus, Sacharomyces cerevisiae*
Hossain *et al.* 2022	Malaysia	*Tor tambroides*	*Enterococcus faecalis* 2674, *Aeromonas* sp. A8-29, *Enterococcus faecalis* FC11682
Asaduzzaman *et al.* 2018	Malaysia	*Tor tambroides*	*Bacillus* sp. AHG22*, Alcaligenes* sp. AFG22*, Shewanell* sp. AFG21
Maas *et al.* 2021	Netherlands	*Oreochromis niloticus*	*Bacillus amyloliquefaciens*
Adeshina *et al.* 2020	Nigeria	*Cyprinus carpio*	*Lactobacillus acidophilus*
Ullah *et al.* 2018	Pakistan	*Cirrhinus mrigala*	*Bacillus subtilis, Sacharomycess cerevisiae*
Chaudhary *et al.* 2021	Pakistan	*Labeo rohita*	*Bacillus subtilis* AsCh-A4
Ramos *et al.* 2017	Portugal	*Oreochromis niloticus*	*Enterococcus* sp., *Bacillus* sp., *Pediococcus sp., Lactobacilli* sp.
Hasan *et al.* 2021	Republic of Korea	*Paralichthys olivaceus*	*Bacillus* sp. SJ-10*, Lactobacillus plantarum* KCCM 11322
Giri *et al.* 2021	Republic of Korea	*Cyprinus carpio*	*Lactobacillus plantarum* L7, *Lactobacillus reuteri* P16
Rahimnejad *et al.* 2018	Republic of Korea	*Sebastes schlegeli*	*Pediococcus acidilactici*
Rhee *et al.* 2020	Republic of Korea	*Paralichthys olivaceus*	*Bacillus subtilis, Groenewaldozyma salmanticensis, Gluconacetobacter liquefaciens*
Gisbert *et al.* 2013	Spain	*Oncorhynchus mykiss*	*Bacillus cereus var toyoi*
Wu *et al.* 2021	Taiwan	*Oreochromis niloticus*	*Bacillus safensis* NPUST1
Meidong *et al.* 2017	Thailand	*Clarias geriepinus* x *C. macrocephalus*	*Bacillus siamensis* B44v
Doan *et al.* 2020	Thailand	*Oreochromis niloticus*	*Lactobacillus plantarum* CR1T5
Doan *et al.* 2016	Thailand	*Oreochromis niloticus*	*Lactobacillus plantarum* CR1T5
Adeoye *et al.* 2016	Thailand	*Oreochromis niloticus*	*Bacillus subtilis, Bacillus licheniformis, Bacillus pumilus*
Doan *et al.* 2021a	Thailand	*Oreochromis niloticus*	*Lactobacillus plantarum* CR1T5
Doan *et al.* 2021b	Thailand	*Oreochromis niloticus*	*B. subtilis* TISTR001, *B. megaterium* TISTR067, *B. licheniformis* DF001
Doan *et al.* 2017	Thailand	*Oreochromis niloticus*	*Lactobacillus plantarum*
Meidong *et al.* 2018	Thailand	*Pangasius bocourti*	*Bacillus aerius* B81e
Doan *et al.* 2018	Thailand	*Oreochromis niloticus*	*Bacillus velezensis* H3.1, *Lactobacillus plantarum* N11
Bunnoy *et al.* 2019	Thailand	*Clarias macrocephalus*	*Acinetobacter* KU011TH
Waiyamitra *et al.* 2020	Thailand	*Oreochromis* spp.	*Bacillus* spp.
Pinpimai *et al.* 2015	Thailand	*Oreochromis niloticus*	*Saccharomyces cerevisiae* JCM 7255

Table 2. The result of meta-analysis from the probiotics effect to the fish growth parameters

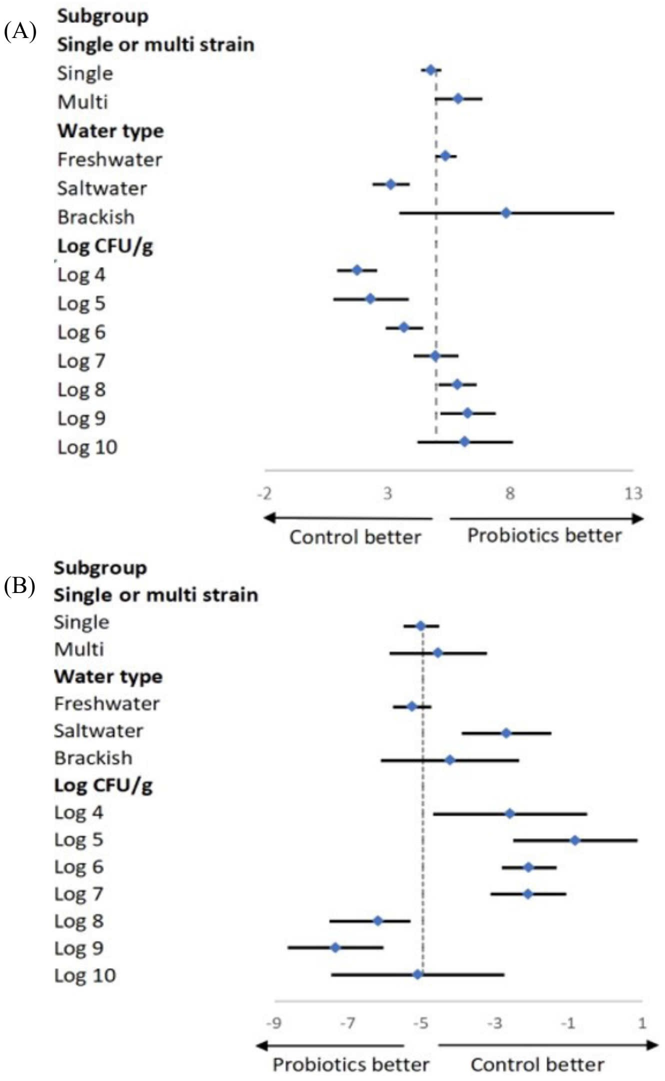
Parameter	Unit	NC	Cumulative effect size (d++)			Std. Error	p-Value	τ^2	Q	Het. p-value	I^2 (%)	N_{fs}
			Estimate	Lower bound	Upper bound							
SGR	%	195	4.988	4.615	5.362	0.191	<0.001	6.751	20797.413	<0.001	99.067	759045R
FCR	Ratio	182	-4.954	-5.428	-4.481	0.242	<0.001	10.223	26505.829	<0.001	99.317	283918R

Nc: Number of comparison; Std. Error: standard error; τ^2: variance of the effect size parameters across the study populations; Q: weighted sum of squared deviations; Het p-value: p-value for heterogeneity; I2: heterogeneity level between studies; N_{fs}: fail-safe number; SGR: specific growth rate; FCR: feed converting ratio; R: model is robust (N_{fs} > 5N+10)

The analysis indicated that the compared studies exhibited high heterogeneity. The compared studies strongly suggested that probiotics have a significant influence on the two tested parameters. This was obtained from the fail-safe number (N_{fs}), which indicates the influence of unpublished studies on the meta-analysis effect size.

In addition to the overall analysis of the effect of probiotics on SGR and FCR, subgroup analyses were conducted to investigate the influence of probiotics on several variables. The variables analyzed included the number of probiotic strains added to the fish feed, type of water environment in which the fish live, and concentration of probiotics added in CFU/g. The first variable examined was the number of strains added to fish feed. There was no significant difference in the effects of single- and multi-strain probiotics on SGR and FCR in this subgroup analysis (Figure 2). The results indicate that the administration of both single strains and multistrains is beneficial for application in aquaculture.

The second variable analyzed was the influence of fish type living in three different water environments: freshwater, saltwater, and brackish water. The addition of probiotics to the feed of freshwater fish was significantly more effective in increasing SGR compared to saltwater and brackish water fish (Figure 2A). Similarly, better results were obtained for FCR when probiotics were administered to freshwater fish (Figure 2B). These findings indicate that the application of probiotics in the feed of freshwater fish has a significantly greater impact than in saltwater and brackish water fish. Freshwater aquaculture is generally more widely practiced than marine or coastal aquaculture. The final variable analyzed in this subgroup analysis was the effect of cell concentration used in the experimental fish feed mixture. The results of this subgroup analysis revealed that cell concentrations that significantly influenced SGR ranged from log 7 to log 10 CFU/g, as examined in this meta-analysis (Figure 2A). Conversely, the concentrations that significantly reduced FCR in this



Subgroup	SGR	95% CI	
Single or multi strain			
Single	4,767	4,367	5,168
Multi	5,883	4,917	6,848
Water type			
Freshwater	5,363	4,94	5,785
Saltwater	3,14	2,376	3,904
Brackish	7,833	3,456	12,211
Log CFU/g			
Log 4	1,768	0,951	2,585
Log 5	2,311	0,765	3,858
Log 6	3,677	2,906	4,448
Log 7	4,95	4,035	5,866
Log 8	5,847	5,07	6,624
Log 9	6,267	5,129	7,406
Log 10	6,146	4,208	8,084

Subgroup	FCR	95% CI	
Single or multi strain			
Single	-5,015	-5,499	-4,531
Multi	-4,548	-5,883	-3,214
Water type			
Freshwater	-5,252	-5,766	-4,737
Saltwater	-2,692	-3,911	-1,473
Brackish	-4,222	-6,106	-2,339
Log CFU/g			
Log 4	-2,594	-4,689	-0,499
Log 5	-0,824	-2,514	0,866
Log 6	-2,807	-2,832	-1,342
Log 7	-2,102	-3,134	-1,07
Log 8	-6,176	-7,504	-5,297
Log 9	-7,334	-8,638	-6,029
Log 10	-5,099	-7,447	-2,751

Figure 2. Subgroup forest plot for (A) SGR and (B) FCR. The dashed lines on the forest plot indicate the cumulative effect size values for the tested parameter

meta-analysis ranged from log 8 to log 10 CFU/g (Figure 2B). These findings provide a recommendation for the minimum cell concentration that significantly affects SGR and FCR, namely, log 8 CFU/g.

DISCUSSION

Meta-analysis is a valuable tool for analyzing existing data from various publication sources to generate comprehensive conclusions. The objective of conducting this meta-analysis on fish growth parameters is to determine whether, among the numerous published studies, the administration of probiotics to fish indeed has a positive impact on their growth. This is intriguing because some publications have yielded results suggesting that probiotic supplementation has an insignificant effect on growth, whereas others have shown that administering probiotics in fish feed significantly influences fish growth. This discrepancy could be influenced by various factors such as fish species, fish age, probiotic concentration, probiotic type, duration of probiotic administration, and others. In this meta-analysis, we focused on the number of probiotic strains, type of aquatic environment in which the fish live, and probiotic concentration.

In this meta-analysis, the comparison of strain numbers was categorized into two groups: single-strain and multistrain. In the context of a single strain, only one probiotic strain is added to the fish feed, whereas in multi-strains, two or more probiotic strains are used in the fish feed. These results indicated that the number of probiotic strains had a positive influence on fish growth and feed efficiency. However, there was no significant difference between the single- and multi-strain approaches for the two growth factors analyzed. The most frequently utilized probiotic strains in the analyzed articles originated from the LAB group. LAB, which are lactic acid bacteria, represent a group of bacteria with promising probiotic potential. This stems from their physiological processes, yielding various compounds during metabolism that exert beneficial effects on the host, including short-chain fatty acids, amines, bacteriocins, vitamins, and exopolysaccharides (Deng *et al.* 2021; Wang *et al.* 2021c; Sadiq 2022). Based on these findings, it is advisable to consider both single- and multi-strain approaches when adding strains to fish feed, as both strategies are effective for the examined growth parameters. Nonetheless, it should be noted that the application of single- or multi-strain probiotics could potentially influence other aspects of fish health and development (Sumon *et al.* 2022).

Based on the articles we found, it is clear that most research on the use of probiotics in fish diets focuses on freshwater fish farming, while only a small portion is dedicated to marine and coastal fish farming. In terms of numbers, freshwater fish farming is significantly more common than marine fish farming (FAO 2020). This preference for freshwater fish farming is understandable, as it offers affordable and readily available aquatic food for a large number of consumers. This differs from marine fish farming, which tends to cater to wealthier customers on a global scale (Zhang *et al.* 2022b). The popularity of freshwater fish farming has been linked to its importance in certain regions. For example, over the last two decades, there has been a noticeable increase in freshwater fish farming across South and Southeast Asia, including Thailand, Myanmar (Belton and Filipski 2019), Vietnam (Loc *et al.* 2010) , Bangladesh (Hernandez *et al.* 2018), and India (Belton *et al.* 2017). In 2018, freshwater aquaculture produced an impressive 51.3 million tons of aquatic animals were produced in freshwater aquaculture, whereas coastal aquaculture and marine culture yielded 30.8 million tons (FAO 2020). Among the fish species covered in the articles, Nile tilapia has been the most studied. Globally, Nile tilapia ranks third in terms of the most cultivated fish species, trailing behind grass and silver carp. Nile tilapia are farmed in more than 80 countries, with major producers including China, Indonesia, Egypt, Brazil, and Thailand (Norman-López and Bjørndal 2009; FAO 2022).

The concentration of probiotic cells that significantly enhanced fish growth and improved feed efficiency in this meta-analysis was log 8 CFU/g. This finding holds significance for individuals engaged in the fishery industry, as both fish growth and feed efficiency are pivotal factors influencing production costs. The positive effect of probiotic feed supplementation offers a potential strategy for feeding practices. Such feeding strategies in aquaculture are important to ensure sustainable production (Araujo *et al.* 2022).

In this context, it is important to note that this meta-analysis had limitations. It exclusively focused on analyzing the two parameters of fish growth and did not delve into other factors influencing fish sustainability, such as metabolic processes, gene expression, and immune system activity. The influence of probiotics on physiological processes in fish is crucial when considering probiotic feed supplementation. Generally, probiotic feeding can stimulate the fish immune system, potentially reducing the risk of mortality [Liu *et al.* 2017; Wang *et al.* 2019; Waiyamitra *et al.* 2020; Doan *et al.* 2021a). However, these aspects were not addressed in this meta-analysis. In the future, it would be advisable to conduct analyses considering other factors that could impact not only fish growth and development, but also overall fish health, thus contributing to sustainability in aquaculture. The meta-analysis concluded that the supplementation of probiotics in fish diets had a positive impact on the two growth factors analyzed. Overall, probiotic supplementation improved the specific growth rate (SGR) and reduced the feed converting ratio (FCR). These findings provide valuable information regarding the enhancement of feeding efficiency in fish by probiotic administration. These effects were shown to be significant in a large number of studies, indicating a strong influence of probiotics on these two tested parameters in aquaculture. This meta-analysis serves as a recommendation for aquaculture industry practitioners to utilize probiotics in their production processes.

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