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Meta-analyses indicate that dietary probiotics significantly improve growth, immune response, and disease resistance in tilapia

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Abstract

Aquaculture is expanding globally, but intensive rearing conditions can cause diseases due to microbial infections leading to financial losses. Probiotics can be used in tilapia farming instead of antibiotics to improve fish health, growth and are environmentally friendly. We provide a synthesis (meta-analysis) of empirical findings to increase statistical power and produce strong evidence for well-informed decision-making with respect to the use of pro, pre, and synbiotics in tilapia culture. In this meta-analysis, we investigated the effects of dietary probiotics on the immune response modulation and resistance of tilapia against common diseases. A systematic search was performed, and the data from 67 studies were included for review and meta-analysis. Pre-challenge, dietary probiotics led to a significant increase ($p < 0.001$) in the weight gain and specific growth rate, a significant decrease ($p < 0.001$) in the feed conversion ratio, but a non-significant increase in survival ($p > 0.05$). However, probiotics significantly ($p < 0.001$) enhanced innate immunity based on serum lysozyme, phagocytotic activity, and phagocytotic index, while disease resistance (survival) of tilapia against pathogenic challenge was also significantly ($p < 0.001$) enhanced. From this study, three categories of probiotics were identified such as single-strain probiotics (SSP), multi-strain probiotics (MSP), and symbiotics (S). The use of synbiotics (probiotics + prebiotics) showed the best effect compared to other probiotic categories, however, more research is needed to ascertain the superiority of synbiotics over other categories. This study concluded that probiotics generally contribute significantly to the growth, immunity, and disease resistance of tilapia.

Keywords Beneficial bacteria · Fish survival · Systematic review · Tilapia · Pathogen · *Lactobacillus sp.*

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Introduction

The worldwide aquaculture business has seen a rise in the importance of using cutting-edge nutritional techniques to improve fish health and disease resistance in recent years (Reverter et al. 2021). Among these approaches, the addition of dietary probiotics has emerged as a viable strategy to support disease resistance and immunological response in a variety of fish species. One of the most popularly raised freshwater fish in the world is tilapia (*Oreochromis* sp.) (Menaga and Fitzsimmons 2017; Prabu et al. 2019), which has considerable economic significance and has been extensively used in experimental trials to ascertain the effectiveness of probiotics. Of the available tilapia species, Nile tilapia (*Oreochromis niloticus*) is the most important due to its ability to feed on a wide variety of food items, tolerance to a wide range of environmental conditions, fast growth rate, and high economic value (Wang and Lu 2016; Abarike et al. 2018a).

The intensified culture of Nile tilapia has led to a fourfold increase in its production which is expected to increase further over the next decade (Chen et al. 2018; Kuebutornye et al. 2020a). However, rapid growth in the aquaculture sector is threatened by many endemic and emerging bacterial diseases, which are caused by *Aeromonas*, *Streptococcus*, and *Flavobacterium* (Basri et al. 2020; Junior et al. 2020; Kerddee et al. 2020; Pauzi et al. 2020; Sookchaiyaporn et al. 2020; Eissa et al. 2021) among others. More recently, the Tilapia Lake Virus has been on the rampage (Dong et al. 2020; Waiyamitra et al 2020). For example, in relatively warm seasons, the death rate of tilapia due to streptococcosis might reach 50% to 70%, while infected tilapia display symptoms like erratic swimming patterns, exophthalmos, and meningitis (Wang et al. 2023). More so, red tilapia fry is very vulnerable to *Flavobacterium columnare* infection with up to 100% cumulative death occurring within 24 h of infection (Van Hai, 2015).

While antibiotics have traditionally been used to improve on-farm biosecurity, there is a need for a paradigm shift in dealing with aquaculture biosecurity risks due to issues concerning antimicrobial residues and antibiotic resistance. This is highlighted by reports from Kumar et al. (2016), and Kavitha et al. (2018). One of the emerging approaches to dealing with these risks is the use of probiotics. Probiotics are defined as live microorganisms that confer health benefits to the host when administered in adequate amounts (Chaudhari and Dwivedi 2022). In aquaculture, probiotics have been used to improve the growth performance, feed conversion efficiency, and disease resistance of fish (Mugwanya et al. 2022). Probiotics are believed to enhance the immune response of fish by stimulating the production of cytokines, immunoglobulins, and other immune-related molecules. Furthermore, probiotics can inhibit the growth of pathogenic bacteria by producing antimicrobial substances or by competing for nutrients and adhesion sites (Dawood et al. 2020; Rohani et al. 2022).

In addition to probiotics, prebiotics are non-digestible food components, such as fructooligosaccharides and inulin, that selectively stimulate the growth and/or activity of beneficial bacteria in the gut, such as *Bifidobacterium* and *Lactobacillus*. Prebiotics can enhance the gut barrier function, promote the production of short-chain fatty acids (SCFAs), and modulate the immune system (Pujari and Banerjee 2021; Rose et al. 2021). Synbiotics are a combination of probiotics and prebiotics that have a synergistic effect on the gut microbiota and the host's health. The prebiotic component in synbiotics provides a substrate for the growth and activity of the probiotic component, while the probiotic component enhances the effectiveness of the prebiotic component by competing for the nutrients and adhesion sites with other microorganisms (Palai et al. 2020; Butt et al. 2021; Zawistowska-Rojek and Tyski 2022). Synbiotics can improve the survival and colonization of probiotics in the gut, enhance the production of SCFAs, and modulate the immune system (Gu et al. 2022). The consideration of synbiotics which are products of pre and probiotics stems from the possibility of demonstrating enhanced potency in protecting fish from diseases because of the synergistic effect of combining both of them.

Several studies have investigated the effects of beneficial materials including plant products (Lin et al. 2019), microalgae (Awad et al. 2022), diatoms (Ayoub et al. 2019), probiotics (Li et al. 2019), prebiotics (Amphan et al. 2019), and synbiotics (Mohammadi et al. 2022) on the immune response and disease resistance of fish species challenged with different pathogens. However, concerning probiotics, prebiotics, and synbiotics, many studies have reported significant positive impacts, while some of them have reported non-significant negative impacts and in some cases, non-significant positive impacts leaving a question as to whether these materials are reliably effective. Therefore, the results require further analysis to evaluate the overall effect and effectiveness of probiotics of different categories on the immune response modulation and disease resistance of targeted commercially important fish species like tilapia following a challenge with important pathogens.

The role of probiotics, prebiotics, and synbiotics in tilapia aquaculture as leading candidates for food sustainability was reviewed by Mugwanya et al. (2022), while Kuebutornye et al. (2020a) narratively reviewed the role of probiotics in the modulation of nutrient utilization, growth, and immunity of tilapia. They concluded that the use of probiotics, prebiotics, and synbiotics in tilapia aquaculture holds a lot of promise, and determining the ideal dosage and time of administration of individual species is crucial for improving growth and immunity. To fill in the existing information gap through quantitative analysis, we carefully chose studies that looked at the effects of dietary probiotics on immune response and disease resistance in tilapia. We then did a systematic review and meta-analysis of the pertinent scientific literature. To find the answers to questions in a research area, a systematic review tries to compile all accessible empirical studies systematically, and this is usually followed by a meta-analysis, the statistical method of analyzing and combining data from numerous related studies (Ahn and Kang 2018).

We sought to present an evidence-based synthesis of the cumulative findings, explaining the genuine potential of dietary probiotics in this context by using tight inclusion criteria and thorough statistical analysis (meta-analysis). The objectives are, therefore, 1) to give an overview of the various probiotics used in enhancing the immune response and disease resistance in tilapia culture, 2) to assess the effects of these probiotics on survival, growth, and nutrient utilization, and 3) to evaluate the contribution of dietary probiotics to immune response and disease resistance of tilapia challenged with common pathogens.

The first objective is crucial to understand the range of probiotic interventions in tilapia aquaculture. This is to examine the different probiotic strains and to identify which strains of probiotics are optimal for enhancing tilapia's immune system and disease resistance. The second objective looks at the value of adding probiotics to aquaculture by analyzing how they affect tilapia survival, growth, and nutrient utilization, while the third objective is critical in establishing the overall effectiveness of probiotics as a disease management strategy. This meta-analysis may be crucial for farmers seeking environmentally friendly methods to contain disease outbreaks, use fewer antibiotics, and boost tilapia populations' resilience to prevalent infections.

Materials and methods

Inclusion and exclusion criteria

In this study, inclusion criteria were applied to published articles not limited to any time. The articles considered were empirical, peer-reviewed publications written in English, which involved the experimental testing of the modulatory effects of different categories of dietary treatments given to different growth stages of tilapia which were later challenged with pathogenic bacteria and included a group that did not receive the probiotics. To maintain the scope of this meta-analysis, we excluded articles that focused on other dietary inclusions, involving other fish species, tilapia exposed to other stressors before administering dietary probiotics (to avoid bias), and studies where fish were not challenged intraperitoneally (to rule out the possibility of bias due to the method of fish challenge). More so, studies with research designs not in line with the objectives of this work were excluded. Furthermore, this study focused on only probiotics (single or multi-strain) and synbiotics and if a study reported more than one probiotic or synbiotic, each was treated independently for the meta-analysis. More so, during data extraction, some studies were excluded from the meta-analysis of certain parameters for failing to provide some relevant information that could be used in the analysis.

Literature search and databases

To reduce the error rate, the search for articles from each database was conducted by one individual at two different periods following the same procedures. The first search which commenced on 3rd January was completed on the 17th of January 2023, while the second (confirmatory) search was completed on the 18th of February 2023. New articles generated from the second search were also screened based on their abstracts and full texts before inclusion. Table 1 summarizes the themes, keywords, and synonyms related to the dietary treatments based on PICO (Population, Intervention, Comparator, and Outcome).

We used Google Scholar (https://scholar.google.com/), Scopus (https://www.scopus.com/), Science Direct (https://www.sciencedirect.com/), Wiley (https://onlinelibrary.wiley.com/), SpringerLink (https://link.springer.com/), and ProQuest (https://www.proquest.com/). In addition, we applied snowballing as a search strategy by checking the reference list of some of the most relevant articles retrieved from other databases (Chen et al. 2020).

Table 1 Search keywords based on PICO headings

S/N	PICO theme	Keywords	Synonyms
1	Population	tilapia	tilapia, Nile tilapia, Mossambique tilapia, *Oreochromis niloticus*
2	Intervention	dietary supplementation	probiotics, synbiotics, beneficial microorganisms
3	Intervention	challenge infection	exposure, disease, pathogen, health
4	Comparator	control	no probiotic fish, non-infected fish, placebo-receiving fish`
5	Outcome	resistance	resistance, performance, immune response, immunomodulation, immunostimulant, immunostimulation, susceptibility, survival, growth

The search was carried out, commencing from 3rd January 2023, in Google Scholar starting with basic keywords which include tilapia, probiotics, and pathogens. After each search with a combination of the keywords in different ways including the use of the advanced search tool, articles that meet the set criteria based on the relevance of their titles were added to the Google Scholar Library. Apart from this, synonyms of the basic keywords such as tilapia, probiotics, synbiotics, challenge infection, exposure, resistance, and immunomodulation (Table 1) were combined in different ways using the advanced search option to bolden the chances of getting the most relevant articles. Apart from Google Scholar, other databases were also systematically searched using different combinations of the keywords based on PICO, while the reference list of some of the articles was assessed for possibly getting new studies.

Identification, screening, and selection

Apart from snowballing, results from each search database were exported mainly as CSV files to Rayyan QCRI online database (https://www.rayyan.ai/) (Ouzzani et al. 2016; Disner et al. 2021). for duplicate removal, the inclusion of relevant publications and exclusion of publications that did not fulfill the inclusion criteria in two steps. The title and abstract screening with Rayyan took place from 7th February 2023 to 16th February 2023.

Data extraction and synthesis

After the screening steps were completed in Rayyan, the resulting publication titles were downloaded as full texts for further review. Full-text articles were critically reviewed and information was extracted from them systematically using Microsoft Office Excel. Before the full-text screening, abstracts of publications that had not been screened in Rayyan were also screened separately. During the full-text screening and information synthesis, some articles were found not to fulfill the inclusion criteria and subsequently excluded from the study. Data extraction from downloaded articles started on 2nd March 2023. Relevant information was tabulated and analyzed descriptively using Google Sheets. General information about the included studies was extracted including citation, probiotic category, tilapia species, experimental duration, fish weight, challenge pathogen, challenge dose, and challenge duration.

Meta-analyses

The meta-analysis was conducted per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page et al. 2021). The following information was extracted: weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), probiotic species, immune response parameters (phagocytic activity, phagocytic index, and serum lysozyme activity), and disease resistance parameter (survival rate) from the included studies.

Meta-analyses were conducted using Review Manager (RevMan) version 5.3 software. Data from eligible studies were extracted and analyzed, including the calculation of and assessment of heterogeneity and effect size statistics based on the I-squared (I^2) and Cochran's Q statistics. The Cochran's Q test determines if the studies included in the meta-analysis have statistically significant heterogeneity. There may be heterogeneity if the Q statistic is substantial. A higher I^2 indicates more heterogeneity among the included research. The relationships between the administered dietary probiotics survival level (pre- and post-challenge) were determined using the odds ratio (OR) with 95% confidence intervals (CIs) for dichotomous data and the standardized mean difference (SMD) with 95% CIs for continuous data.

The confidence interval (CI) is a statistical metric that provides a range of values within which the real or population effect size is anticipated to fall with a specific level of confidence. Based on data from the included research, it assesses the level of uncertainty surrounding the anticipated impact magnitude. The confidence interval is typically shown as a range of values with an associated confidence level, which is frequently set at 95%. The Mantel-Haenszel method is used by RevMan 5 to calculate effect estimates for binary outcomes, while the inverse variance approach for continuous outcomes. Pooled estimates were presented using the forest plot. In some studies, different categories and species of probiotics were tested, and these were treated individually, although the same information was used for the control. To standardize the survival data, the number of individuals that survived as used in the meta-analyses was extrapolated from each study based on the percentage survival/mortality reported and the total number of fish used in the experiment for both control and treatment groups. Only studies that reported this information were included in the meta-analyses. As for other parameters, the mean and standard deviation values were extracted and used for the meta-analysis. For the post-challenge survival, meta-analysis was focused on the intraperitoneal challenge, and no other routes such as oral, immersion, and intramuscular. Apart from cumulative percentage mortality, which was considered as a measure, some other publications applied the relative level of protection (RLP) and were excluded from the meta-analysis on post-challenge survival to ensure reliability.

To further ascertain the impact of the probiotic treatments, Pearson's correlation was used to check if there existed a relationship between the post-challenge survival level and the pre-challenge feed conversion ratio. In addition, to determine the probiotic type that performed most effectively to enhance survival in fish following challenge with pathogens, survival data were compared for the three groups of probiotics including single-strain probiotics, multi-strain probiotics, and synbiotic (prebiotics + probiotics). Because the data was generally not normally distributed, we used a non-parametric test such as the Kruskal Wallis test using IBM SPSS version 26. In studies with more than one concentration of the treatment probiotic, a treatment that was identified as the best for growth and nutrient utilization, immunity, and disease resistance was chosen as the subject of comparison with the control. More so, in cases where the challenge concentration was in more than one treatment, the treatment with the highest concentration was chosen for comparison. In cases where different durations of dietary probiotics were administered, the duration closer to those of other studies was selected as in the case of Aly et al. (2008a) where two months was selected as against one or eight months. Also, where different densities of tilapia were used as in Mohammadi et al. (2022), the density closer to what is generally obtainable in other studies was selected.

Results

Included studies

We employed four databases and search engines, as well as the snowballing method, to find relevant articles. This search yielded a total of 399 records, which were uploaded to

Rayyan (http://www.www.rayyan.ai) for the identification and elimination of duplicates and ineligible articles. After eliminating duplicates and ineligible articles, we were left with 113 articles from the four databases and two articles from the snowballing method. Out of these 115 articles, 29 articles were excluded for not meeting inclusion criteria, and the remaining 84 studies were assessed for eligibility. Eventually, 66 articles were deemed eligible and used for data extraction. These articles covered 118 unique cases where probiotics were used as single-strain probiotics (SSP), multi-strain probiotics (MSP), or synbiotics (in combination with prebiotic materials). Please refer to Fig. 1 for a visual representation of this process.

Summary of studies

Overall, the trend in the number of included studies exhibits relative stability from 2006 to 2014, followed by a gradual increase from 2015 to 2019 with the highest number reported for 2021 (Fig. 2). The mean weight of tilapia reported ranges from 0.20 g to 100 g. Most of the mean weights fall within the range of 1.5 g to 60 g. The most common probiotic category used in the studies is SSP, while most of the feeding experiments were carried out for 56 days and ranged from 21 to 112 days. Generally, the challenge pathogens mentioned in the table include *Aeromonas hydrophila, Pseudomonas florescence, Streptococcus iniae, Streptococcus agalactiae, Enterococcus faecalis, Aeromonas sobria, Vibrio parahaemolyticus, Yersinia ruckeri, Edwardsiella tarda, Pseudomonas aeruginosa* and TILV (Tilapia Lake Virus). Among the pathogens, *A. hydrophila, S. iniae*, and *S. agalactiae* of different concentrations were the most frequently reported pathogens, while the most reported challenge duration was 14 days (Table 2).

Meta-analysis

The data extracted from the studies included in this analysis were evaluated for several parameters such as weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), pre-challenge survival, serum lysozyme (SL), phagocytotic index (PI), phagocytotic activity (PA), and post-challenge survival. These were the parameters common to most of the studies.

Analysis of all continuous data showed a significant effect of probiotics and synbiotics in the diet of tilapia on WG (SMD = 3.63; 95% CI 3.11 to 4.15; $p < 0.001$; $I^2 = 99\%$) (Fig. 3), SGR (SMD = 2.61; 95% CI 2.04 to 3.19; $p < 0.001$; $I^2 = 99\%$) (Fig. 4), and FCR (SMD = −0.36; 95% CI -0.36 to −0.36; $p < 0.001$; $I^2 = 100\%$) (Fig. 5). However, there was no significant effect on the pre-challenge survival (OR = 1.28, 95% CI 0.94 to 1.74; $p = 0.05$; $I^2 = 50\%$) (Fig. 6) which was based on a dichotomous analysis. As per the immunomodulatory effects, Fig. 7 shows that there was a significant effect of probiotics and synbiotics on SL (SMD = 5.59; 95% CI 4.36 to 6.82; $p < 0.001$; $I^2 = 100\%$), PI (SMD = 9.34; 95% CI 7.23 to 11.64; $p < 0.001$; $I^2 = 99\%$), and PA (SMD = 2.61; 95% CI 0.90 to 4.33; $p = 0.03$; I2 = 98%), respectively. More so, for disease resistance, the post-challenge survival showed a significant effect (SMD = 5.02; 95% CI 3.98 to 6.35; $p < 0.001$; $I^2 = 71\%$) (Fig. 8) of the probiotic on tilapia. Based on the Kruskal-Wallis's test there was no significant difference ($p > 0.05$) in the performance of different categories of probiotics assessed.

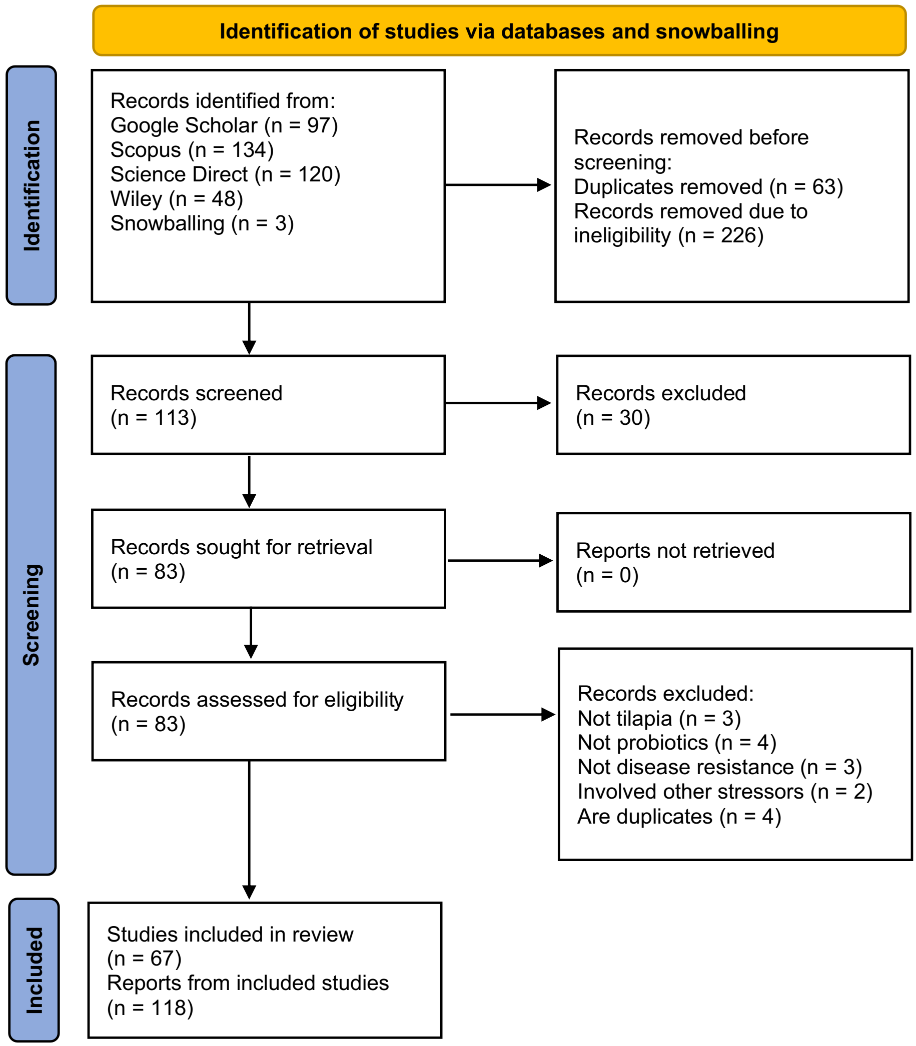


Fig. 1 PRISMA flow chart for included studies

A total of 99 cases with available data on survival post-challenge survival and probiotic types were used for comparison and Kruskal-Wallis H was 2.807, df $=2$, and not significant at $p=0.208$. However, descriptively, the synbiotic category recorded the highest mean rank (66.94), while the multi-strain probiotic recorded the lowest mean rank (46.66). Serum lysozyme and phagocytotic activity were reported to be significantly higher ($p<0.05$) in most other assessed studies. However, these were not included in the meta-analysis since the data was not available for inclusion in our meta-analysis. According to Pearson's correlation, there was a non-significantly ($p=0.587$) weak negative ($r=-0.630$) correlation between the post-challenge cumulative percentage survival and the FCR.

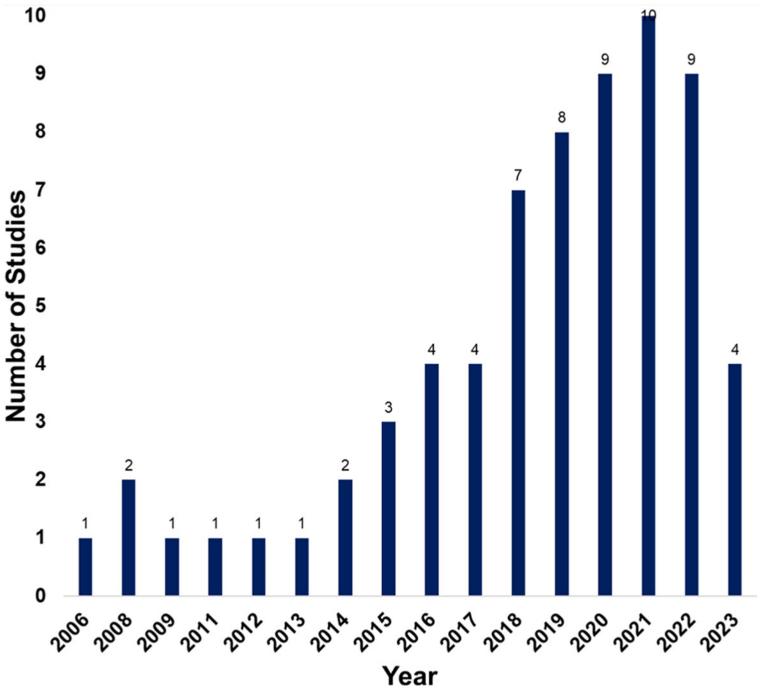


Fig. 2 Number of included studies per year

Discussion

The findings of this study provide valuable insights into the potential of dietary probiotics as an effective tool to enhance the health and productivity of fish species, particularly tilapia. By modulating the gut microbiota, improving nutrient utilization, and stimulating the immune system, probiotics have a significant impact on the health and disease resistance of fish (Rohani et al. 2022). The most used probiotics in tilapia were *Bacillus* spp. especially *B. subtilis* and *Lactobacillus* spp., especially *L. plantarum* (Supplementary material 1). However, the optimal dose, duration, and combination of probiotics for maximum benefit to fish health and production remain unclear. Therefore, further research is needed to explore these factors.

In line with the outcome of this meta-analysis that dietary probiotics significantly improve weight gain, specific growth rate, and feed conversion ratio in tilapia, other fish species including mud carp (*Cirrhinus molitorella*), common carp (*Cyprinus carpio*), freshwater catfishes (*Gulsa tengra* and *Mystus cavasius*) among many others (Gupta et al. 2014; Banu et al. 2020; Yu et al. 2022) that have been reported. It is however worth noting that meta-analyses of those studies have not been found. From this meta-analysis, there was a significant decrease in the feed conversion ratio, and this is supported by the meta-analysis of Toledo et al. (2019) who reported a significant impact of probiotics on the SGR and FCR of shrimps. However, the non-significant positive effect of dietary probiotic inclusion on pre-challenge survival contradicts the report of Toledo et al. (2019). This may be because tilapia generally and particularly *O. niloticus*, which occurred most in this analysis, is known to be a rugged fish with a high level of stress tolerance which may be which

Table 2 Summary of reviewed studies with probiotic categories (PC), experimental duration (ED), weight of fish used (W), challenge pathogens with concentration, and challenge duration (CD)

Citation	PC	Tilapia species	ED (days)	W (g) (mean ± SD)	Challenge pathogen (concentration)	CD
Abomughaid (2020)	2 SSP	*O. niloticus*	56	30 ± 10	*A. hydrophila* (10^8 bacteria/L)	7
Abass et al. (2018)	SSP	*O. niloticus*	84	21.4	*A. hydrophila* (2×10^8 CFU/mL)	14
Abdel-Tawwab (2012)	SSP	*O. niloticus*	84	0.25–0.48	*A. hydrophila* (5×10^5 CFU/mL)	10
Abu-Elala et al. (2013)*	1 SSP and 1 S	*O. niloticus*	21	80 ± 5	*A. hydrophila* (2×10^8 CFU/ mL)	7
Addo et al. (2017)*	2 SSP and 2 S	*O. niloticus*	56	7.47 ± 0.11	*A. hydrophila* (3.9×10^7 CFU/fish)	NS
Aly et al. (2008a, b)*	2 SSP and 1 MSP, 1SSP	*O. niloticus*	56	5.2 ± 0.9, 6.5 ± 1.0	*A. hydrophila* (10^8 cells/mL)	15, NS
Aly et al. (2008a)*	2 SSP and 1 MSP	*O. niloticus*	56	5.2 ± 0.9	*P. florescence* (10^8 cells/mL)	15
Aly et al. (2008a)*	2 SSP and 1 MSP	*O. niloticus*	56	5.2 ± 0.9	*S. iniae* (10^8 cells/mL)	15
Ammar et al. (2022)	MSP2	*O. niloticus*	84	25.57 ± 0.30	*A. hydrophila* (3.16×10^7 CFU/mL)	10
Cavalcante et al. (2020)	MSP3 and 2 S	*O. niloticus*	63	8.84 ± 1.29	*A. hydrophila* (1.26×10^8 CFU/mL)	15
Chen et al. (2019)	SSP	*O. niloticus*	56	5.53 ± 0.45	*A. hydrophila* (1.0×10^6 CFU/fish)	7
Chen et al. (2019)	SSP	*O. niloticus*	56	5.53 ± 0.45	*S. iniae* (1.0×10^5 CFU/fish)	7
Dawood et al. (2020)	SSP and S	*O. niloticus*	90	15.94 ± 0.02	*A. hydrophila* (2.8×10^6 CFU/fish)	10
de Moraes et al. (2022)	MSP2 and SSP	*O. niloticus*	45	2.61 ± 0.02	*A. hydrophila* (2.1×10^8 CFU/fish)	10
Gewaily et al. (2021)	SSP	*O. niloticus*	30	28.21 ± 1.34	*A. hydrophila* (2×10^8 CFU/mL)	10
Hamdan et al. (2016)	SSP	*O. niloticus*	40	24.5 ± 0.26	*A. hydrophila* (5×10^7 CFU/mL)	14
Iwashita et al. (2015)	MSP3	*O. niloticus*	42	25.00 ± 0.05	*A. hydrophila* (2×10^6 CFU/mL)	21
Iwashita et al. (2015)	MSP3	*O. niloticus*	42	25.00 ± 0.05	*S. iniae* (10^4 CFU/mL)	21
Jose et al. (2023)	MSP2	*O. niloticus*	60	11.17 ± 1.18	*A. hydrophila* (2×10^7 CF/mL)	10
Kaew-on et al. (2016)*	2 SSP and 1 MSP2	*O. niloticus*	42	0.68 ± 0.02	*A. hydrophila* (5×10^9 CFU/ mL)	7
Kuebutornye et al. (2020b)*	3 SSP and 1 MSP3	*O. niloticus*	28	46.24 ± 0.48	*A. hydrophila* (1×10^8 CFU/ mL)	7
Li et al. (2019)	SSP	*O. niloticus*	55	56.21 ± 0.81	*S. agalactiae* (1.5×10^6 CFU/mL)	14
Li et al. (2019)	SSP	*O. niloticus*	56	56.21 ± 0.81	*A. hydrophila* (1.5×10^6 CFU/mL)	14
Mohammadi et al. (2021)*	SSP and MSP5	*O. niloticus*	112	8.63 ± 3.35	*A. hydrophila* (10^8 cells/mL)	7
Mohammadi et al. (2022)*	S and SSP	*O. niloticus*	56	11.05 ± 0.23	*A. hydrophila* (10^7 cells/mL)	10

Table 2 (continued)

Citation	PC	Tilapia species	ED (days)	W (g) (mean ± SD)	Challenge pathogen (concentration)	CD
Samson et al. (2022)	3 SSP	*O. niloticus*	30	1.76 ± 0.07	*A. hydrophila* (10^7 CFU/mL)	14
Saputra et al. 2016)	SSP	*O. niloticus*	56	1.49 ± 0.15	*A. hydrophila* (10^6 CFU/fish)	7
Suprayudi et al. (2017)	SSP	*O. niloticus*	56	11.07 ± 0.07	*A. hydrophila* (10^6 cells/mL)	14
Tan et al. (2019)	SSP	*O. niloticus*	56	4.10 ± 0.34	*A. hydrophila* (10^6 cells/fish)	7
Tan et al. (2019)	SSP	*O. niloticus*	56	4.10 ± 0.34	*S. iniae* (10^5 cells/fish)	7
Xu et al. (2022)	SSP	*O. niloticus*	70	1.68 ± 0.05	*A. hydrophila* (2.5×10^6 CFU/g)	7
Abarike et al. (2018b)	1 MSP2	*O. niloticus*	28	53.01 ± 1.00	*S. agalactiae* (1×10^9 CFU/mL)	14
Abarike et al. (2018a)	S	*O. niloticus*	28	57 ± 2	*S. agalactiae* (1×10^9 CFU/mL)	14
Abd El-Rhman et al. (2009)*	2 SSP and 1 MSP2	*O. niloticus*	90	2.35 ± 0.10	*A. hydrophila* (0.3×10^7 cells/ mL)	14
Abou-El-Atta et al. (2019)*	1 SSP and 1 S	*O. niloticus*	60	15.2 ± 0.6	*A. sobria* (5×10^5 CFU/ mL)	10
Addo et al. (2016)	SSP	*O. niloticus*	21	16.5 ± 0.2	*S. iniae* (8×10^6 CFU/fish)	16
Bocamdé et al. (2020)	SSP	*O. niloticus*	60	10.99 ± 1.10	*V. parahaemolyticus* (10^5 CFU/mL)	21
Büyükdeveci et al. (2023)*	2 SSP	*O. niloticus*	60	52	*S. iniae* (7×10^3 CFU/ mL)	21
Dowidar et al. (2018)*	1MSP 6 and 2 SSP	*O. niloticus*	56	24.00 ± 0.02	*A. hydrophila* (4×10^8 CFU/ mL)	14
Eissa & AbouElGheit (2014)	SSP	*O. niloticus*	45	2.93 ± 0.22	*A. hydrophilla* (1.8×10^8 CFU/ mL)	7
El-Nobi et al. (2021)	S	*O. niloticus*	75	18 ± 2	*P. aeruginosa* (3×10^7 cells/mL)	15
Gobi et al. (2018)	SSP	*O. mossambicus*	28	24.0 ± 2.5	*A. hydrophila* (10^7 cells/mL)	10
Han et al. (2015)	SSP	*O. niloticus*	70	3.83 ± 0.03	*S. iniae* (10^9 CFU/ mL)	5
Khunrang et al. (2021)	MSP	*O. niloticus*	45	17.18 ± 2.12	*S. agalactiae* (10^7 CF/mL)	14
Li et al. (2020)	SSP	*O. niloticus*	56	56.23 ± 0.64	*S. agalactiae* (1.5×10^6 CFU/mL)	14
Liu et al. (2017)	SSP	*O. niloticus*	28	95 ± 8	*S. agalactiae* (1.5×10^6 CFU/ mL)	14
Liu et al. (2021)*	4 MSP2	*O. mossambicus*	42	99.17 ± 1.80	*S. agalactiae* (5.13×10^4 CFU/mL)	7
Mousa et al. (2021)	SSP	*O. niloticus*	70	20 ± 5	*E. faecalis* (3×10^7 CFU/mL)	14
Moustafa et al. (2021)	MSP 3	*O. niloticus*	NS	100 ± 5	*S. iniae* (10^6 CFU/ mL)	15
Ng et al. (2014)*	2 SSP and 1 MSP2	Red hybrid tilapia	23	4.2 ± 0.1	*S. agalactiae* (1.9 × 107 CFU/ mL)	23

Table 2 (continued)

Citation	PC	Tilapia species	ED (days)	W (g) (mean ± SD)	Challenge pathogen (concentration)	CD
Panase et al. (2023)	SSP	*O. niloticus*	56	24.5 ± 1.6	*S. agalactiae* (1 × 10^8 CFU/ mL)	14
Guimarães et al. (2022)	MSP	*O. niloticus*	49	20.00 ± 2.07	*S. agalactiae* (not stated)	14
Dias et al. (2022)*	2 SSP and 1 MSP	*O. niloticus*	90	6.71 ± 0.93	*S. agalactiae* (1.7 × 10^7 CFU/g)	4
Sewaka et al. (2019)*	1 SSP and 1 S	*O. niloticus*	28	14.05 ± 0.42	*A. veronii* (10^7 CFU/fish)	15
Sherif et al. (2021)	SSP	*O. niloticus*	28	60 ± 5	*E. tarda* (2.54 × 10^6 CFU/mL)	14
Sookchaiyaporn et al. (2020)	SSP	*O. niloticus*	28	2	*S. agalactiae* (10^8 CFU/mL)	14
Srisapoome & Areechon (2017)	SSP	*O. niloticus*	30	50	*S. agalactiae* (1 × 10^8 CFU/mL)	14
Mohamed et al. (2019)*	1 S and 1 S	*O. niloticus*	84	4.00	*A. hydrophila* (10^7 cells/mL)	15
Waiyamitra et al. (2020)	SSP	Red hybrid tilapia	21	10.0 ± 0.5	TILV (10^5 TCID 50/mL)	28
Shelby et al. (2006)	NS	*O. niloticus*	83	0.29	*S. iniae* (10^6 CFU/fish)	NS
Suphoronski et al. (2021)	SSP	*O. niloticus*	38	11.93 ± 0.59	*S. agalactiae* (8.8 × 10^5 CFU/mL)	30
Doan et al. (2018)*	3 SSP and 1 MSP	*O. niloticus*	30	21.80 ± 0.03	*S. agalactiae* (10^7 CFU/mL)	15
Wu et al. (2021)	SSP	*O. niloticus*	56	0.550 ± 0.042	*S. iniae* (10^5 CFU/g)	7
Xia et al. (2018)*	2 SSP and 1 MSP 2	*O. niloticus*	42	0.20 ± 0.05	*S. agalactiae* (1 × 10^5 CFU/mL)	14
Xia et al. (2020)*	2 SSP and 1 MSP2	*O. niloticus*	42	0.20 ± 0.05	*S. agalactiae* (1 × 10^5 CFU/mL)	14
Zhu et al. (2019)	SSP	GIFT tilapia	42	46.91 ± 0.17	*S. agalactiae* (10^8 CFU/ mL)	14
Santos et al. (2023)	MSP2	*O. niloticus*	54	5.76 ± 0.24	*S. agalactiae* (1.7 × 10^7 CFU/g)	15
Selim & Reda (2015)	2 SSP	*O. niloticus*	30	27.70 ± 0.22	*Y. ruckeri* (1.5 × 10^8 cells/ mL)	14
Sîrbu et al. (2022)	MSP	*O. niloticus*	40	1.50 ± 0.01	*A. hydrophila* (1.3 × 109 CFU/mL)	21
Sîrbu et al. (2022)	MSP	*O. niloticus*	40	1.50 ± 0.01	*P. fluorescens* (1.5 × 109 CFU/mL)	21
Wulansari et al. (2019)	SSP	Red hybrid tilapia	NS	13.85 ± 1.02	*A. hydrophila* (varying)	14
Sayed et al. (2011)	SSP	*O. niloticus*	70	5.000 ± 0.218	*A. hydrophila* (5 × 10^5 cells/mL)	15
Liao et al. (2022)*	SSP	*O. niloticus*	56	100 ± 15	*S. agalactiae* (1 × 10^8 CFU/mL)	7

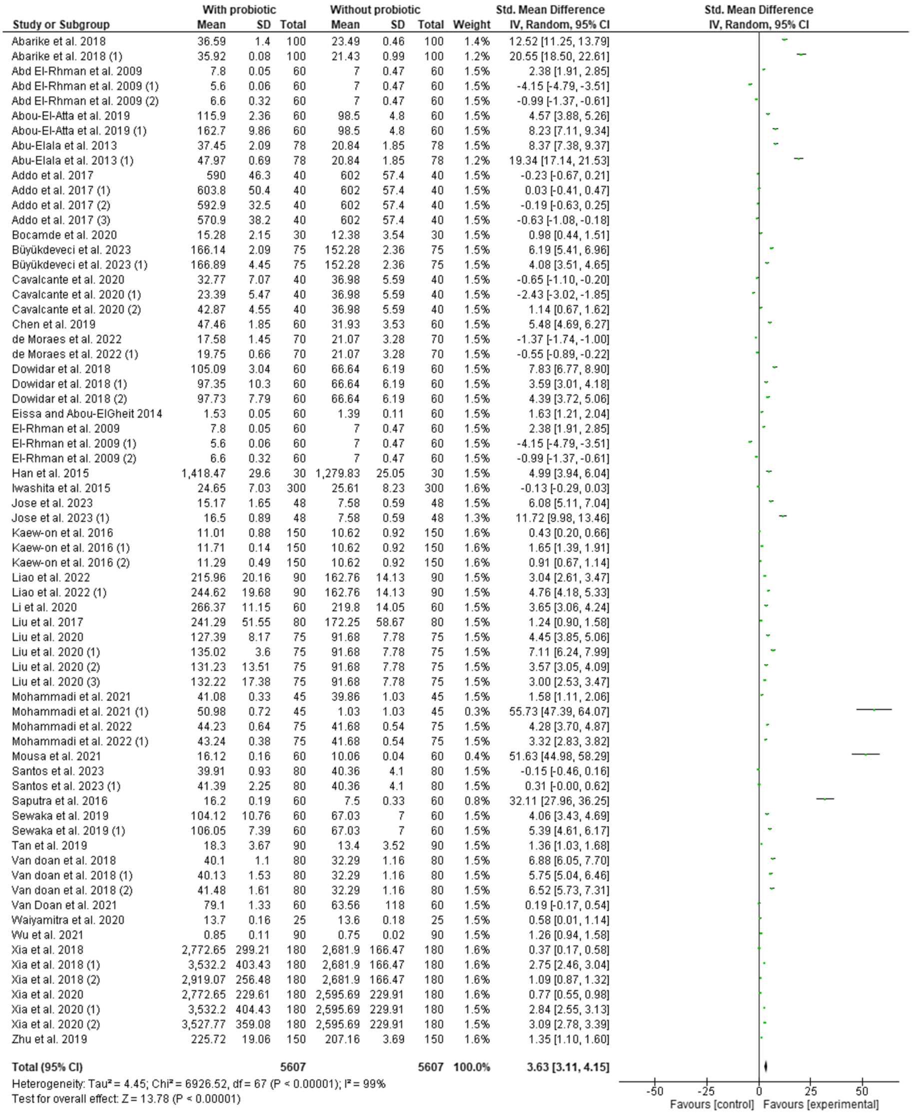
SSP single strain probiotic, *MSP* multi-strain probiotics, *S* synbiotic, *Number prefix* number of individual probiotics used, *number suffix for MSP* number of probiotic strains used. * = denotes studies where more than a single probiotic type was used but fish was challenged with the same pathogen after the experiment may lead to a lack of significant reduction in the survival level of the control compared to the treated group (Camargo-dos-Santos et al. 2021).

The positive effects of probiotics on the immune response modulation and disease resistance of fish have been attributed to their ability to modulate the gut microbiota and enhance the production of immune-related molecules, such as cytokines, immunoglobulins, and lysozyme (Merrifield et al. 2010; Zhou et al. 2019). This can potentially reduce the need for antibiotics or other chemical treatments, and this aligns with the growing global concern about the development of antibiotic resistance in aquaculture. Probiotics can effectively stimulate the phagocytes in the host and improve phagocytic activity, especially by lactic acid bacteria (LAB) probiotics like *L. rhamnosus*, *L. acidophilus*, and *L. lactis*, as observed in several animals (Akhter et al. 2015; Zhu and Su 2022). In line with the findings of this meta-analysis, oral administration of *Clostridium butyricum* bacteria in rainbow trout (*Onchorychus mykiss)* increased their phagocytosis activity (Sakai et al. 1995). In rainbow trout, an increase in phagocytic activity was observed after four weeks, with the highest activity against *V. anguillarum* seen in trout two weeks after starting the probiotic feeding mode (Sharifuzzaman and Austin 2009; Akhter et al. 2015).

In line with the outcome of fish survival pre-challenge which showed a significant effect of administered probiotics, survival post-challenge recorded a significant effect. This establishes the effectiveness of these probiotics in disease resistance. Probiotics can directly inhibit the growth and colonization of pathogenic bacteria by producing antimicrobial substances, competing for nutrients and adhesion sites, and modulating the host's immune response (Khaneghah et al. 2020; Wang et al. 2021; Liu et al. 2022). Probiotics can also indirectly stimulate the host's immune response by modulating the gut microbiota composition and enhancing the gut barrier function, which can influence the overall health and disease resistance of the fish (Zhang et al. 2016; Wuertz et al. 2021; Guangxin et al. 2022).

Despite the lack of significant difference in the survival of tilapia post-challenge based on the type of probiotic administered, synbiotics seem to be the best option. It also presupposes that symbiotics are likely the most effective. Therefore, we recommend more empirical research on the use of multi-strain probiotics and synbiotics. More so, this will pave the way for more powerful analysis which could give better insight. Supplementary material 1 shows a list of multi-strain probiotics and synbiotics that have given promising results. The possible reason for the better performance of synbiotics compared to SSP, MSP may result from the fact that synbiotics have a synergistic effect, meaning that the combined action of the probiotics and prebiotics provides greater health benefits than the sum of their individual effects (Butt et al. 2021). This synergistic effect can lead to better digestive health, immune function, and other health benefits (Yousefi et al. 2023). Synbiotics can modulate the gut microbiota more effectively than probiotics or prebiotics alone (Hijová 2022). This is because probiotics and prebiotics can have different effects on the gut microbiota and combining them can lead to a more balanced and diverse gut microbiota.

To create a multi-strain probiotic that is optimized for desired outcomes, it is important to carefully select the strains of bacteria and their concentrations, as well as the mode of administration. Using a mixture design allows for the study of interactions among microorganisms to improve the effectiveness of the probiotic. A multi-strain probiotic offers advantages over a single-strain probiotic, as different strains can work together synergistically to improve immune function and digestive health. The development of a multi-strain probiotic requires consideration of various factors, including the ability of the strains to survive digestion and colonize the gut, as well as their production of beneficial compounds. Additionally, the mode of administration plays a role in the effectiveness of the probiotic (Melo-Bolívar et al. 2021). Ultimately, a well-designed multi-strain probiotic can help improve the health and growth performance of the fish. However, more research is needed to fully understand the mechanisms behind the superior performance of synbiotics.

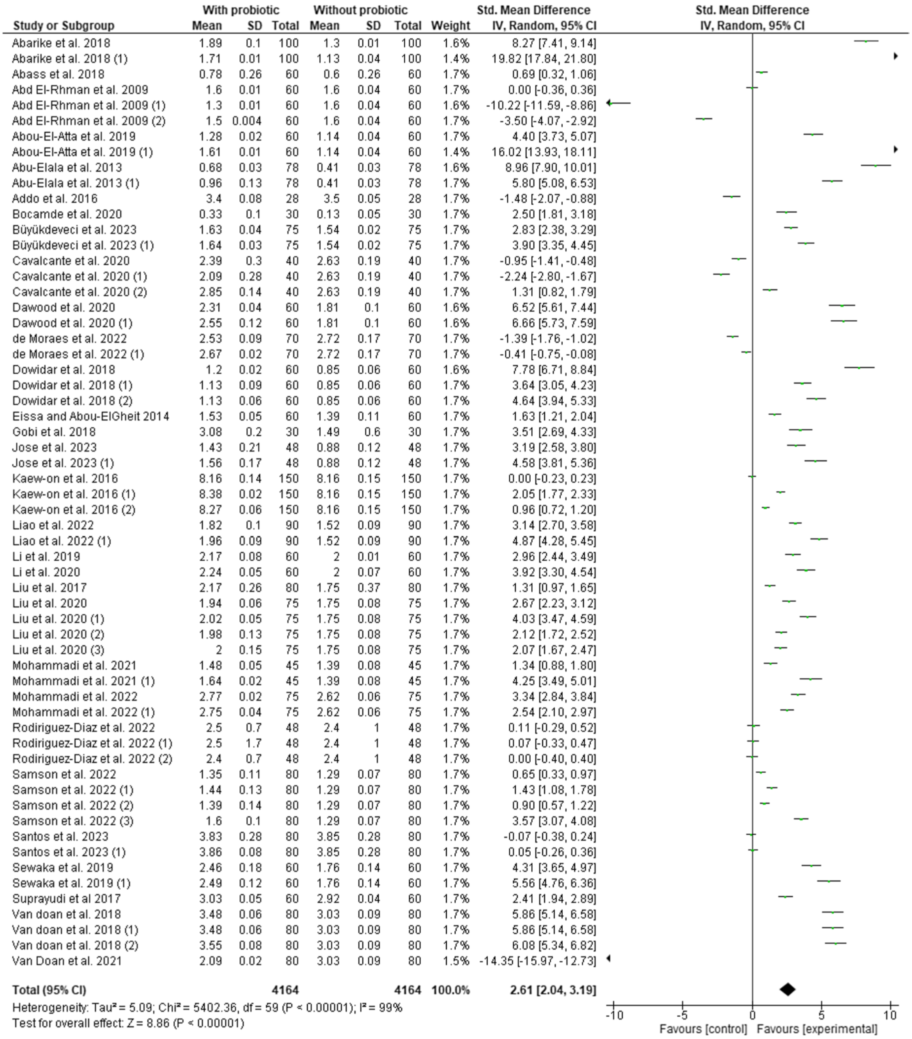


Study or Subgroup	With probiotic			Without probiotic				Std. Mean Difference
	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
Abarike et al. 2018	36.59	1.4	100	23.49	0.46	100	1.4%	12.52 [11.25, 13.79]
Abarike et al. 2018 (1)	35.92	0.08	100	21.43	0.99	100	1.2%	20.55 [18.50, 22.61]
Abd El-Rhman et al. 2009	7.8	0.05	60	7	0.47	60	1.5%	2.38 [1.91, 2.85]
Abd El-Rhman et al. 2009 (1)	5.6	0.06	60	7	0.47	60	1.5%	-4.15 [-4.79, -3.51]
Abd El-Rhman et al. 2009 (2)	6.6	0.32	60	7	0.47	60	1.5%	-0.99 [-1.37, -0.61]
Abou-El-Atta et al. 2019	115.9	2.36	60	98.5	4.8	60	1.5%	4.57 [3.88, 5.26]
Abou-El-Atta et al. 2019 (1)	162.7	9.86	60	98.5	4.8	60	1.5%	8.23 [7.11, 9.34]
Abu-Elala et al. 2013	37.45	2.09	78	20.84	1.85	78	1.5%	8.37 [7.38, 9.37]
Abu-Elala et al. 2013 (1)	47.97	0.69	78	20.84	1.85	78	1.2%	19.34 [17.14, 21.53]
Addo et al. 2017	590	46.3	40	602	57.4	40	1.5%	-0.23 [-0.67, 0.21]
Addo et al. 2017 (1)	603.8	50.4	40	602	57.4	40	1.5%	0.03 [-0.41, 0.47]
Addo et al. 2017 (2)	592.9	32.5	40	602	57.4	40	1.5%	-0.19 [-0.63, 0.25]
Addo et al. 2017 (3)	570.9	38.2	40	602	57.4	40	1.5%	-0.63 [-1.08, -0.18]
Bocamde et al. 2020	15.28	2.15	30	12.38	3.54	30	1.5%	0.98 [0.44, 1.51]
Büyükdeveci et al. 2023	166.14	2.09	75	152.28	2.36	75	1.5%	6.19 [5.41, 6.96]
Büyükdeveci et al. 2023 (1)	166.89	4.45	75	152.28	2.36	75	1.5%	4.08 [3.51, 4.65]
Cavalcante et al. 2020	32.77	7.07	40	36.98	5.59	40	1.5%	-0.65 [-1.10, -0.20]
Cavalcante et al. 2020 (1)	23.39	5.47	40	36.98	5.59	40	1.5%	-2.43 [-3.02, -1.85]
Cavalcante et al. 2020 (2)	42.87	4.55	40	36.98	5.59	40	1.5%	1.14 [0.67, 1.62]
Chen et al. 2019	47.46	1.85	60	31.93	3.53	60	1.5%	5.48 [4.69, 6.27]
de Moraes et al. 2022	17.58	1.45	70	21.07	3.28	70	1.5%	-1.37 [-1.74, -1.00]
de Moraes et al. 2022 (1)	19.75	0.66	70	21.07	3.28	70	1.5%	-0.55 [-0.89, -0.22]
Dowidar et al. 2018	105.09	3.04	60	66.64	6.19	60	1.5%	7.83 [6.77, 8.90]
Dowidar et al. 2018 (1)	97.35	10.3	60	66.64	6.19	60	1.5%	3.59 [3.01, 4.18]
Dowidar et al. 2018 (2)	97.73	7.79	60	66.64	6.19	60	1.5%	4.39 [3.72, 5.06]
Eissa and Abou-ElGheit 2014	1.53	0.05	60	1.39	0.11	60	1.5%	1.63 [1.21, 2.04]
El-Rhman et al. 2009	7.8	0.05	60	7	0.47	60	1.5%	2.38 [1.91, 2.85]
El-Rhman et al. 2009 (1)	5.6	0.06	60	7	0.47	60	1.5%	-4.15 [-4.79, -3.51]
El-Rhman et al. 2009 (2)	6.6	0.32	60	7	0.47	60	1.5%	-0.99 [-1.37, -0.61]
Han et al. 2015	1,418.47	29.6	30	1,279.83	25.05	30	1.5%	4.99 [3.94, 6.04]
Iwashita et al. 2015	24.65	7.03	300	25.61	8.23	300	1.6%	-0.13 [-0.29, 0.03]
Jose et al. 2023	15.17	1.65	48	7.58	0.59	48	1.5%	6.08 [5.11, 7.04]
Jose et al. 2023 (1)	16.5	0.89	48	7.58	0.59	48	1.3%	11.72 [9.98, 13.46]
Kaew-on et al. 2016	11.01	0.88	150	10.62	0.92	150	1.6%	0.43 [0.20, 0.66]
Kaew-on et al. 2016 (1)	11.71	0.14	150	10.62	0.92	150	1.6%	1.65 [1.39, 1.91]
Kaew-on et al. 2016 (2)	11.29	0.49	150	10.62	0.92	150	1.6%	0.91 [0.67, 1.14]
Liao et al. 2022	215.96	20.16	90	162.76	14.13	90	1.5%	3.04 [2.61, 3.47]
Liao et al. 2022 (1)	244.62	19.68	90	162.76	14.13	90	1.5%	4.76 [4.18, 5.33]
Li et al. 2020	266.37	11.15	60	219.8	14.05	60	1.5%	3.65 [3.06, 4.24]
Liu et al. 2017	241.29	51.55	80	172.25	58.67	80	1.5%	1.24 [0.90, 1.58]
Liu et al. 2020	127.39	8.17	75	91.68	7.78	75	1.5%	4.45 [3.85, 5.06]
Liu et al. 2020 (1)	135.02	3.6	75	91.68	7.78	75	1.5%	7.11 [6.24, 7.99]
Liu et al. 2020 (2)	131.23	13.51	75	91.68	7.78	75	1.5%	3.57 [3.05, 4.09]
Liu et al. 2020 (3)	132.22	17.38	75	91.68	7.78	75	1.5%	3.00 [2.53, 3.47]
Mohammadi et al. 2021	41.08	0.33	45	39.86	1.03	45	1.5%	1.58 [1.11, 2.06]
Mohammadi et al. 2021 (1)	50.98	0.72	45	1.03	1.03	45	0.3%	55.73 [47.39, 64.07]
Mohammadi et al. 2022	44.23	0.64	75	41.68	0.54	75	1.5%	4.28 [3.70, 4.87]
Mohammadi et al. 2022 (1)	43.24	0.38	75	41.68	0.54	75	1.5%	3.32 [2.83, 3.82]
Mousa et al. 2021	16.12	0.16	60	10.06	0.04	60	0.4%	51.63 [44.98, 58.29]
Santos et al. 2023	39.91	0.93	80	40.36	4.1	80	1.5%	-0.15 [-0.46, 0.16]
Santos et al. 2023 (1)	41.39	2.25	80	40.36	4.1	80	1.5%	0.31 [-0.00, 0.62]
Saputra et al. 2016	16.2	0.19	60	7.5	0.33	60	0.8%	32.11 [27.96, 36.25]
Sewaka et al. 2019	104.12	10.76	60	67.03	7	60	1.5%	4.06 [3.43, 4.69]
Sewaka et al. 2019 (1)	106.05	7.39	60	67.03	7	60	1.5%	5.39 [4.61, 6.17]
Tan et al. 2019	18.3	3.67	90	13.4	3.52	90	1.5%	1.36 [1.03, 1.68]
Van doan et al. 2018	40.1	1.1	80	32.29	1.16	80	1.5%	6.88 [6.05, 7.70]
Van doan et al. 2018 (1)	40.13	1.53	80	32.29	1.16	80	1.5%	5.75 [5.04, 6.46]
Van doan et al. 2018 (2)	41.48	1.61	80	32.29	1.16	80	1.5%	6.52 [5.73, 7.31]
Van Doan et al. 2021	79.1	1.33	60	63.56	118	60	1.5%	0.19 [-0.17, 0.54]
Waiyamitra et al. 2020	13.7	0.16	25	13.6	0.18	25	1.5%	0.58 [0.01, 1.14]
Wu et al. 2021	0.85	0.11	90	0.75	0.02	90	1.5%	1.26 [0.94, 1.58]
Xia et al. 2018	2,772.65	299.21	180	2,681.9	166.47	180	1.6%	0.37 [0.17, 0.58]
Xia et al. 2018 (1)	3,532.2	403.43	180	2,681.9	166.47	180	1.6%	2.75 [2.46, 3.04]
Xia et al. 2018 (2)	2,919.07	256.48	180	2,681.9	166.47	180	1.6%	1.09 [0.87, 1.32]
Xia et al. 2020	2,772.65	229.61	180	2,595.69	229.91	180	1.6%	0.77 [0.55, 0.98]
Xia et al. 2020 (1)	3,532.2	404.43	180	2,595.69	229.91	180	1.5%	2.84 [2.55, 3.13]
Xia et al. 2020 (2)	3,527.77	359.08	180	2,595.69	229.91	180	1.5%	3.09 [2.78, 3.39]
Zhu et al. 2019	225.72	19.06	150	207.16	3.69	150	1.6%	1.35 [1.10, 1.60]
Total (95% CI)			**5607**			**5607**	**100.0%**	**3.63 [3.11, 4.15]**

Heterogeneity: Tau² = 4.45; Chi² = 6926.52, df = 67 (P < 0.00001); I² = 99%
Test for overall effect: Z = 13.78 (P < 0.00001)

Fig. 3 Forest plot of randomized control trials on the effect of dietary probiotic administration on weight gain (WG) of tilapia (35 studies, 68 records)

There are still some knowledge gaps that need to be addressed in future research. For example, the optimal dosage based on fish size, duration, and administration route of probiotics in tilapia exposed to pathogen challenge is not well-established and may vary depending on the probiotic strain, fish species, and environmental conditions. Therefore, further investigations into these aspects are proposed. The potential interactions between probiotics and other factors, such as diet composition, water quality, and other stressors, also need further investigation. Studies comparing the efficacy of different probiotic strains for specific pathogens will also be beneficial. Finally, there is a need



Study or Subgroup	With probiotic Mean	SD	Total	Without probiotic Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Abarike et al. 2018	1.89	0.1	100	1.3	0.01	100	1.6%	8.27 [7.41, 9.14]
Abarike et al. 2018 (1)	1.71	0.01	100	1.13	0.04	100	1.4%	19.82 [17.84, 21.80]
Abass et al. 2018	0.78	0.26	60	0.6	0.26	60	1.7%	0.69 [0.32, 1.06]
Abd El-Rhman et al. 2009	1.6	0.01	60	1.6	0.04	60	1.7%	0.00 [-0.36, 0.36]
Abd El-Rhman et al. 2009 (1)	1.3	0.01	60	1.6	0.04	60	1.6%	-10.22 [-11.59, -8.86]
Abd El-Rhman et al. 2009 (2)	1.5	0.004	60	1.6	0.04	60	1.7%	-3.50 [-4.07, -2.92]
Abou-El-Atta et al. 2019	1.28	0.02	60	1.14	0.04	60	1.7%	4.40 [3.73, 5.07]
Abou-El-Atta et al. 2019 (1)	1.61	0.01	60	1.14	0.04	60	1.4%	16.02 [13.93, 18.11]
Abu-Elala et al. 2013	0.68	0.03	78	0.41	0.03	78	1.6%	8.96 [7.90, 10.01]
Abu-Elala et al. 2013 (1)	0.96	0.13	78	0.41	0.03	78	1.7%	5.80 [5.08, 6.53]
Addo et al. 2016	3.4	0.08	28	3.5	0.05	28	1.7%	-1.48 [-2.07, -0.88]
Bocamde et al. 2020	0.33	0.1	30	0.13	0.05	30	1.7%	2.50 [1.81, 3.18]
Büyükdeveci et al. 2023	1.63	0.04	75	1.54	0.02	75	1.7%	2.83 [2.38, 3.29]
Büyükdeveci et al. 2023 (1)	1.64	0.03	75	1.54	0.02	75	1.7%	3.90 [3.35, 4.45]
Cavalcante et al. 2020	2.39	0.3	40	2.63	0.19	40	1.7%	-0.95 [-1.41, -0.48]
Cavalcante et al. 2020 (1)	2.09	0.28	40	2.63	0.19	40	1.7%	-2.24 [-2.80, -1.67]
Cavalcante et al. 2020 (2)	2.85	0.14	40	2.63	0.19	40	1.7%	1.31 [0.82, 1.79]
Dawood et al. 2020	2.31	0.04	60	1.81	0.1	60	1.6%	6.52 [5.61, 7.44]
Dawood et al. 2020 (1)	2.55	0.12	60	1.81	0.1	60	1.6%	6.66 [5.73, 7.59]
de Moraes et al. 2022	2.53	0.09	70	2.72	0.17	70	1.7%	-1.39 [-1.76, -1.02]
de Moraes et al. 2022 (1)	2.67	0.02	70	2.72	0.17	70	1.7%	-0.41 [-0.75, -0.08]
Dowidar et al. 2018	1.2	0.02	60	0.85	0.06	60	1.6%	7.78 [6.71, 8.84]
Dowidar et al. 2018 (1)	1.13	0.09	60	0.85	0.06	60	1.7%	3.64 [3.05, 4.23]
Dowidar et al. 2018 (2)	1.13	0.06	60	0.85	0.06	60	1.7%	4.64 [3.94, 5.33]
Eissa and Abou-ElGheit 2014	1.53	0.05	60	1.39	0.11	60	1.7%	1.63 [1.21, 2.04]
Gobi et al. 2018	3.08	0.2	30	1.49	0.6	30	1.7%	3.51 [2.69, 4.33]
Jose et al. 2023	1.43	0.21	48	0.88	0.12	48	1.7%	3.19 [2.58, 3.80]
Jose et al. 2023 (1)	1.56	0.17	48	0.88	0.12	48	1.7%	4.58 [3.81, 5.36]
Kaew-on et al. 2016	8.16	0.14	150	8.16	0.15	150	1.7%	0.00 [-0.23, 0.23]
Kaew-on et al. 2016 (1)	8.38	0.02	150	8.16	0.15	150	1.7%	2.05 [1.77, 2.33]
Kaew-on et al. 2016 (2)	8.27	0.06	150	8.16	0.15	150	1.7%	0.96 [0.72, 1.20]
Liao et al. 2022	1.82	0.1	90	1.52	0.09	90	1.7%	3.14 [2.70, 3.58]
Liao et al. 2022 (1)	1.96	0.09	90	1.52	0.09	90	1.7%	4.87 [4.28, 5.45]
Li et al. 2019	2.17	0.08	60	2	0.01	60	1.7%	2.96 [2.44, 3.49]
Li et al. 2020	2.24	0.05	60	2	0.07	60	1.7%	3.92 [3.30, 4.54]
Liu et al. 2017	2.17	0.26	80	1.75	0.37	80	1.7%	1.31 [0.97, 1.65]
Liu et al. 2020	1.94	0.06	75	1.75	0.08	75	1.7%	2.67 [2.23, 3.12]
Liu et al. 2020 (1)	2.02	0.05	75	1.75	0.08	75	1.7%	4.03 [3.47, 4.59]
Liu et al. 2020 (2)	1.98	0.13	75	1.75	0.08	75	1.7%	2.12 [1.72, 2.52]
Liu et al. 2020 (3)	2	0.15	75	1.75	0.08	75	1.7%	2.07 [1.67, 2.47]
Mohammadi et al. 2021	1.48	0.05	45	1.39	0.08	45	1.7%	1.34 [0.88, 1.80]
Mohammadi et al. 2021 (1)	1.64	0.02	45	1.39	0.08	45	1.7%	4.25 [3.49, 5.01]
Mohammadi et al. 2022	2.77	0.02	75	2.62	0.06	75	1.7%	3.34 [2.84, 3.84]
Mohammadi et al. 2022 (1)	2.75	0.04	75	2.62	0.06	75	1.7%	2.54 [2.10, 2.97]
Rodiriguez-Diaz et al. 2022	2.5	0.7	48	2.4	1	48	1.7%	0.11 [-0.29, 0.52]
Rodiriguez-Diaz et al. 2022 (1)	2.5	1.7	48	2.4	1	48	1.7%	0.07 [-0.33, 0.47]
Rodiriguez-Diaz et al. 2022 (2)	2.4	0.7	48	2.4	1	48	1.7%	0.00 [-0.40, 0.40]
Samson et al. 2022	1.35	0.11	80	1.29	0.07	80	1.7%	0.65 [0.33, 0.97]
Samson et al. 2022 (1)	1.44	0.13	80	1.29	0.07	80	1.7%	1.43 [1.08, 1.78]
Samson et al. 2022 (2)	1.39	0.14	80	1.29	0.07	80	1.7%	0.90 [0.57, 1.22]
Samson et al. 2022 (3)	1.6	0.1	80	1.29	0.07	80	1.7%	3.57 [3.07, 4.08]
Santos et al. 2023	3.83	0.28	80	3.85	0.28	80	1.7%	-0.07 [-0.38, 0.24]
Santos et al. 2023 (1)	3.86	0.08	80	3.85	0.28	80	1.7%	0.05 [-0.26, 0.36]
Sewaka et al. 2019	2.46	0.18	60	1.76	0.14	60	1.7%	4.31 [3.65, 4.97]
Sewaka et al. 2019 (1)	2.49	0.12	60	1.76	0.14	60	1.7%	5.56 [4.76, 6.36]
Suprayudi et al 2017	3.03	0.05	60	2.92	0.04	60	1.7%	2.41 [1.94, 2.89]
Van doan et al. 2018	3.48	0.06	80	3.03	0.09	80	1.7%	5.86 [5.14, 6.58]
Van doan et al. 2018 (1)	3.48	0.06	80	3.03	0.09	80	1.7%	5.86 [5.14, 6.58]
Van doan et al. 2018 (2)	3.55	0.08	80	3.03	0.09	80	1.7%	6.08 [5.34, 6.82]
Van Doan et al. 2021	2.09	0.02	80	3.03	0.09	80	1.5%	-14.35 [-15.97, -12.73]
Total (95% CI)			**4164**			**4164**	**100.0%**	**2.61 [2.04, 3.19]**

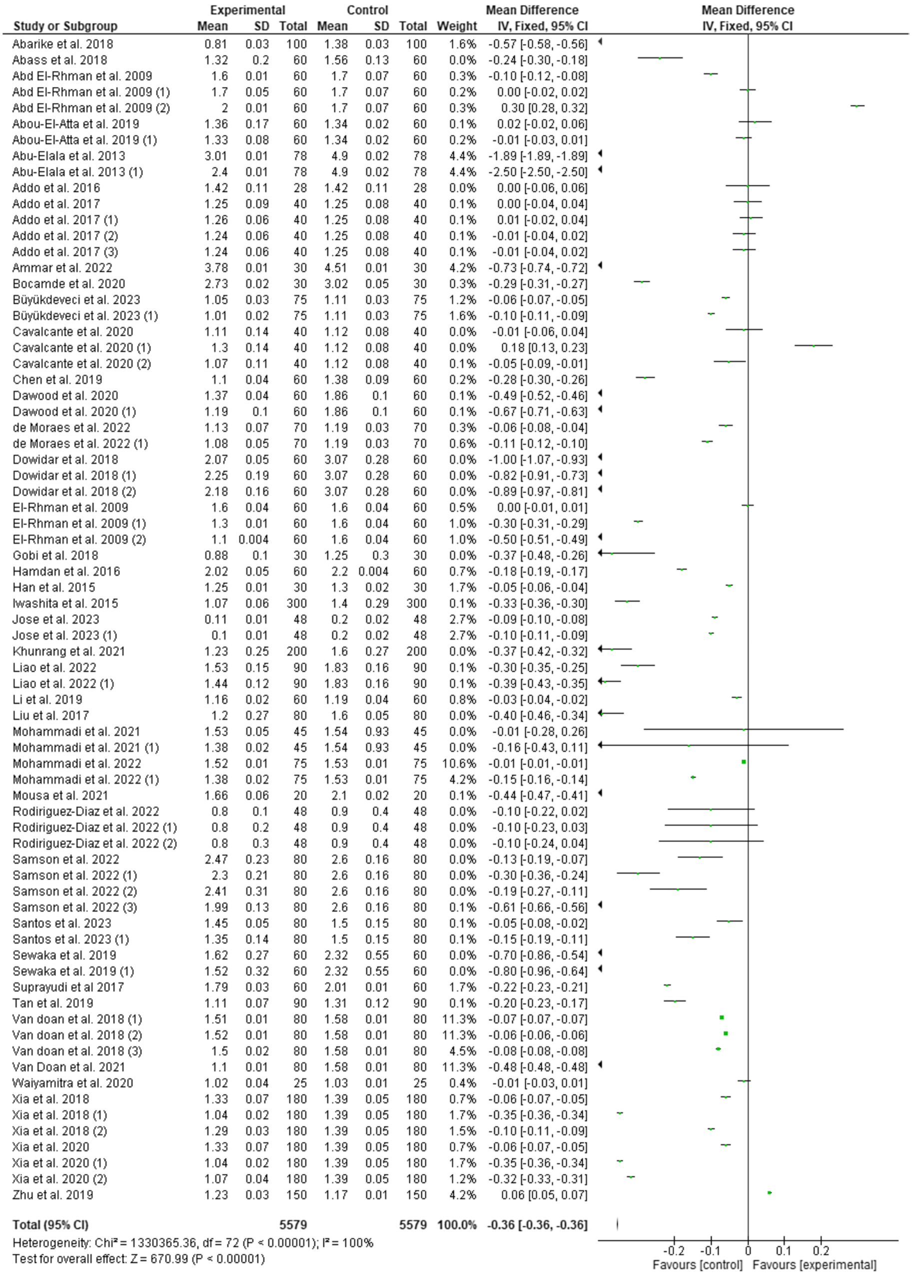
Heterogeneity: $Tau^2 = 5.09$; $Chi^2 = 5402.36$, df = 59 ($P < 0.00001$); $I^2 = 99\%$

Test for overall effect: $Z = 8.86$ ($P < 0.00001$)

Fig. 4 Forest plot of randomized control trials on the effect of dietary probiotic administration on the specific growth rate (SGR) of tilapia (30 studies, 60 records)

for future systematic review and meta-analysis of the outcome for other species and other potentially promising immunostimulants that are also cheap and more environmentally friendly.

It is worth mentioning that, to glean more information, there is a need to intensify research on the impact of varying crude protein levels on the effectiveness of probiotics concerning immunity and disease resistance. In doing this, the issue of cost must also be factored in to ascertain if higher protein levels are worth it. For example, Zheng et al. (2011) concluded that the prebiotic, *GroBiotic®-A* positively influenced the growth performance and feed efficiency of tilapia fed with diets containing 29% crude protein to levels comparable to fish fed the 33% Crude Protein (CP) diet, while Abdel-Tawwab (2012) stated that 45% CP diets performed better with yeast probiotic compared to 35% diets when



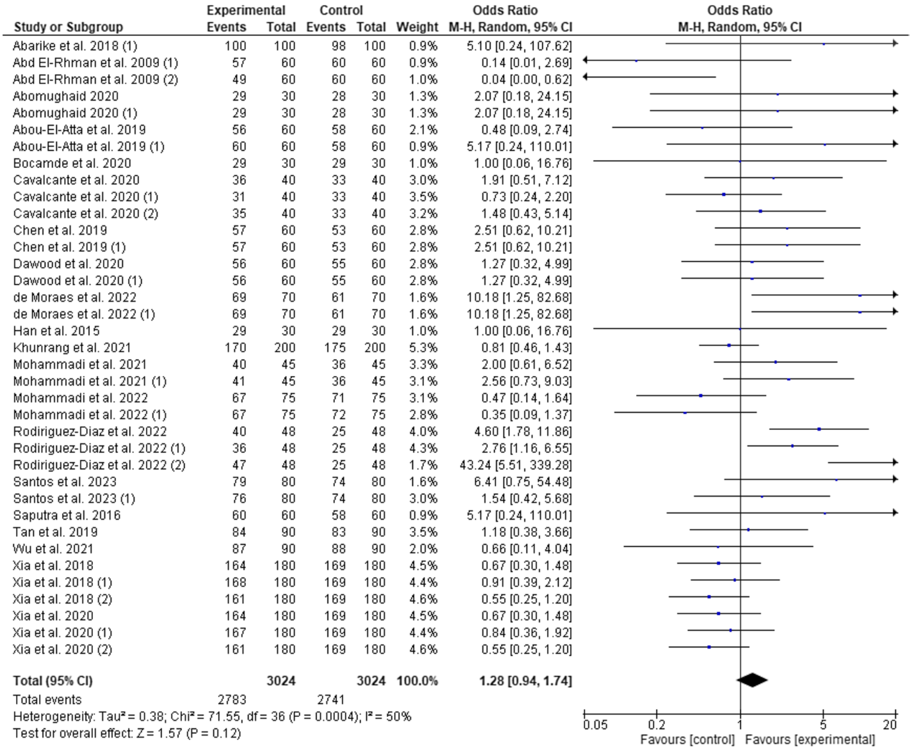
Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Fixed, 95% CI
Abarike et al. 2018	0.81	0.03	100	1.38	0.03	100	1.6%	-0.57 [-0.58, -0.56]
Abass et al. 2018	1.32	0.2	60	1.56	0.13	60	0.0%	-0.24 [-0.30, -0.18]
Abd El-Rhman et al. 2009	1.6	0.01	60	1.7	0.07	60	0.3%	-0.10 [-0.12, -0.08]
Abd El-Rhman et al. 2009 (1)	1.7	0.05	60	1.7	0.07	60	0.2%	0.00 [-0.02, 0.02]
Abd El-Rhman et al. 2009 (2)	2	0.01	60	1.7	0.07	60	0.3%	0.30 [0.28, 0.32]
Abou-El-Atta et al. 2019	1.36	0.17	60	1.34	0.02	60	0.1%	0.02 [-0.02, 0.06]
Abou-El-Atta et al. 2019 (1)	1.33	0.08	60	1.34	0.02	60	0.2%	-0.01 [-0.03, 0.01]
Abu-Elala et al. 2013	3.01	0.01	78	4.9	0.02	78	4.4%	-1.89 [-1.89, -1.89]
Abu-Elala et al. 2013 (1)	2.4	0.01	78	4.9	0.02	78	4.4%	-2.50 [-2.50, -2.50]
Addo et al. 2016	1.42	0.11	28	1.42	0.11	28	0.0%	0.00 [-0.06, 0.06]
Addo et al. 2017	1.25	0.09	40	1.25	0.08	40	0.1%	0.00 [-0.04, 0.04]
Addo et al. 2017 (1)	1.26	0.06	40	1.25	0.08	40	0.1%	0.01 [-0.02, 0.04]
Addo et al. 2017 (2)	1.24	0.06	40	1.25	0.08	40	0.1%	-0.01 [-0.04, 0.02]
Addo et al. 2017 (3)	1.24	0.06	40	1.25	0.08	40	0.1%	-0.01 [-0.04, 0.02]
Ammar et al. 2022	3.78	0.01	30	4.51	0.01	30	4.2%	-0.73 [-0.74, -0.72]
Bocamde et al. 2020	2.73	0.02	30	3.02	0.05	30	0.3%	-0.29 [-0.31, -0.27]
Büyükdeveci et al. 2023	1.05	0.03	75	1.11	0.03	75	1.2%	-0.06 [-0.07, -0.05]
Büyükdeveci et al. 2023 (1)	1.01	0.02	75	1.11	0.03	75	1.6%	-0.10 [-0.11, -0.09]
Cavalcante et al. 2020	1.11	0.14	40	1.12	0.08	40	0.0%	-0.01 [-0.06, 0.04]
Cavalcante et al. 2020 (1)	1.3	0.14	40	1.12	0.08	40	0.0%	0.18 [0.13, 0.23]
Cavalcante et al. 2020 (2)	1.07	0.11	40	1.12	0.08	40	0.1%	-0.05 [-0.09, -0.01]
Chen et al. 2019	1.1	0.04	60	1.38	0.09	60	0.2%	-0.28 [-0.30, -0.26]
Dawood et al. 2020	1.37	0.04	60	1.86	0.1	60	0.1%	-0.49 [-0.52, -0.46]
Dawood et al. 2020 (1)	1.19	0.1	60	1.86	0.1	60	0.1%	-0.67 [-0.71, -0.63]
de Moraes et al. 2022	1.13	0.07	70	1.19	0.03	70	0.3%	-0.06 [-0.08, -0.04]
de Moraes et al. 2022 (1)	1.08	0.05	70	1.19	0.03	70	0.6%	-0.11 [-0.12, -0.10]
Dowidar et al. 2018	2.07	0.05	60	3.07	0.28	60	0.0%	-1.00 [-1.07, -0.93]
Dowidar et al. 2018 (1)	2.25	0.19	60	3.07	0.28	60	0.0%	-0.82 [-0.91, -0.73]
Dowidar et al. 2018 (2)	2.18	0.16	60	3.07	0.28	60	0.0%	-0.89 [-0.97, -0.81]
El-Rhman et al. 2009	1.6	0.04	60	1.6	0.04	60	0.5%	0.00 [-0.01, 0.01]
El-Rhman et al. 2009 (1)	1.3	0.01	60	1.6	0.04	60	1.0%	-0.30 [-0.31, -0.29]
El-Rhman et al. 2009 (2)	1.1	0.004	60	1.6	0.04	60	1.0%	-0.50 [-0.51, -0.49]
Gobi et al. 2018	0.88	0.1	30	1.25	0.3	30	0.0%	-0.37 [-0.48, -0.26]
Hamdan et al. 2016	2.02	0.05	60	2.2	0.004	60	0.7%	-0.18 [-0.19, -0.17]
Han et al. 2015	1.25	0.01	30	1.3	0.02	30	1.7%	-0.05 [-0.06, -0.04]
Iwashita et al. 2015	1.07	0.06	300	1.4	0.29	300	0.1%	-0.33 [-0.36, -0.30]
Jose et al. 2023	0.11	0.01	48	0.2	0.02	48	2.7%	-0.09 [-0.10, -0.08]
Jose et al. 2023 (1)	0.1	0.01	48	0.2	0.02	48	2.7%	-0.10 [-0.11, -0.09]
Khunrang et al. 2021	1.23	0.25	200	1.6	0.27	200	0.0%	-0.37 [-0.42, -0.32]
Liao et al. 2022	1.53	0.15	90	1.83	0.16	90	0.1%	-0.30 [-0.35, -0.25]
Liao et al. 2022 (1)	1.44	0.12	90	1.83	0.16	90	0.1%	-0.39 [-0.43, -0.35]
Li et al. 2019	1.16	0.02	60	1.19	0.04	60	0.8%	-0.03 [-0.04, -0.02]
Liu et al. 2017	1.2	0.27	80	1.6	0.05	80	0.0%	-0.40 [-0.46, -0.34]
Mohammadi et al. 2021	1.53	0.05	45	1.54	0.93	45	0.0%	-0.01 [-0.28, 0.26]
Mohammadi et al. 2021 (1)	1.38	0.02	45	1.54	0.93	45	0.0%	-0.16 [-0.43, 0.11]
Mohammadi et al. 2022	1.52	0.01	75	1.53	0.01	75	10.6%	-0.01 [-0.01, -0.01]
Mohammadi et al. 2022 (1)	1.38	0.02	75	1.53	0.01	75	4.2%	-0.15 [-0.16, -0.14]
Mousa et al. 2021	1.66	0.06	20	2.1	0.02	20	0.1%	-0.44 [-0.47, -0.41]
Rodiriguez-Diaz et al. 2022	0.8	0.1	48	0.9	0.4	48	0.0%	-0.10 [-0.22, 0.02]
Rodiriguez-Diaz et al. 2022 (1)	0.8	0.2	48	0.9	0.4	48	0.0%	-0.10 [-0.23, 0.03]
Rodiriguez-Diaz et al. 2022 (2)	0.8	0.3	48	0.9	0.4	48	0.0%	-0.10 [-0.24, 0.04]
Samson et al. 2022	2.47	0.23	80	2.6	0.16	80	0.0%	-0.13 [-0.19, -0.07]
Samson et al. 2022 (1)	2.3	0.21	80	2.6	0.16	80	0.0%	-0.30 [-0.36, -0.24]
Samson et al. 2022 (2)	2.41	0.31	80	2.6	0.16	80	0.0%	-0.19 [-0.27, -0.11]
Samson et al. 2022 (3)	1.99	0.13	80	2.6	0.16	80	0.1%	-0.61 [-0.66, -0.56]
Santos et al. 2023	1.45	0.05	80	1.5	0.15	80	0.1%	-0.05 [-0.08, -0.02]
Santos et al. 2023 (1)	1.35	0.14	80	1.5	0.15	80	0.1%	-0.15 [-0.19, -0.11]
Sewaka et al. 2019	1.62	0.27	60	2.32	0.55	60	0.0%	-0.70 [-0.86, -0.54]
Sewaka et al. 2019 (1)	1.52	0.32	60	2.32	0.55	60	0.0%	-0.80 [-0.96, -0.64]
Suprayudi et al 2017	1.79	0.03	60	2.01	0.01	60	1.7%	-0.22 [-0.23, -0.21]
Tan et al. 2019	1.11	0.07	90	1.31	0.12	90	0.1%	-0.20 [-0.23, -0.17]
Van doan et al. 2018 (1)	1.51	0.01	80	1.58	0.01	80	11.3%	-0.07 [-0.07, -0.07]
Van doan et al. 2018 (2)	1.52	0.01	80	1.58	0.01	80	11.3%	-0.06 [-0.06, -0.06]
Van doan et al. 2018 (3)	1.5	0.02	80	1.58	0.01	80	4.5%	-0.08 [-0.08, -0.08]
Van Doan et al. 2021	1.1	0.01	80	1.58	0.01	80	11.3%	-0.48 [-0.48, -0.48]
Waiyamitra et al. 2020	1.02	0.04	25	1.03	0.01	25	0.4%	-0.01 [-0.03, 0.01]
Xia et al. 2018	1.33	0.07	180	1.39	0.05	180	0.7%	-0.06 [-0.07, -0.05]
Xia et al. 2018 (1)	1.04	0.02	180	1.39	0.05	180	1.7%	-0.35 [-0.36, -0.34]
Xia et al. 2018 (2)	1.29	0.03	180	1.39	0.05	180	1.5%	-0.10 [-0.11, -0.09]
Xia et al. 2020	1.33	0.07	180	1.39	0.05	180	0.7%	-0.06 [-0.07, -0.05]
Xia et al. 2020 (1)	1.04	0.02	180	1.39	0.05	180	1.7%	-0.35 [-0.36, -0.34]
Xia et al. 2020 (2)	1.07	0.04	180	1.39	0.05	180	1.2%	-0.32 [-0.33, -0.31]
Zhu et al. 2019	1.23	0.03	150	1.17	0.01	150	4.2%	0.06 [0.05, 0.07]
Total (95% CI)			**5579**			**5579**	**100.0%**	**-0.36 [-0.36, -0.36]**

Heterogeneity: Chi² = 1330365.36, df = 72 (P < 0.00001); I² = 100%
Test for overall effect: Z = 670.99 (P < 0.00001)

Fig. 5 Forest plot of randomized control trials on the effect of dietary probiotic administration on the feed conversion ratio (FCR) of tilapia (40 studies and 73 records)

Saccharomyces cerevisiae was included in the diet of Nile tilapia, whereas, most studies in this review used CP levels between 29 and 41%.

Apart from growth or disease resistance, probiotics e.g., *Saccharomyces* have also helped with stress resistance in fish. For example, Abass et al. (2018) recorded a higher



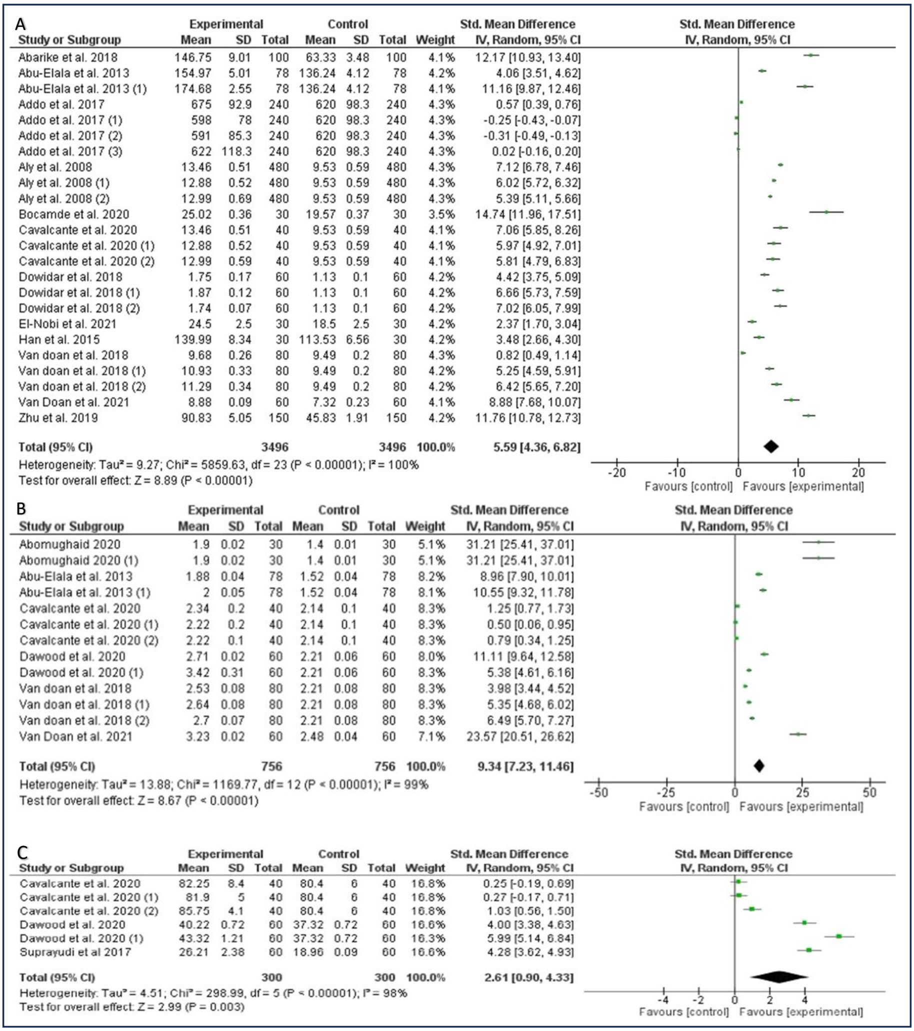
Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
Abarike et al. 2018 (1)	100	100	98	100	0.9%	5.10 [0.24, 107.62]
Abd El-Rhman et al. 2009 (1)	57	60	60	60	0.9%	0.14 [0.01, 2.69]
Abd El-Rhman et al. 2009 (2)	49	60	60	60	1.0%	0.04 [0.00, 0.62]
Abomughaid 2020	29	30	28	30	1.3%	2.07 [0.18, 24.15]
Abomughaid 2020 (1)	29	30	28	30	1.3%	2.07 [0.18, 24.15]
Abou-El-Atta et al. 2019	56	60	58	60	2.1%	0.48 [0.09, 2.74]
Abou-El-Atta et al. 2019 (1)	60	60	58	60	0.9%	5.17 [0.24, 110.01]
Bocamde et al. 2020	29	30	29	30	1.0%	1.00 [0.06, 16.76]
Cavalcante et al. 2020	36	40	33	40	3.0%	1.91 [0.51, 7.12]
Cavalcante et al. 2020 (1)	31	40	33	40	3.5%	0.73 [0.24, 2.20]
Cavalcante et al. 2020 (2)	35	40	33	40	3.2%	1.48 [0.43, 5.14]
Chen et al. 2019	57	60	53	60	2.8%	2.51 [0.62, 10.21]
Chen et al. 2019 (1)	57	60	53	60	2.8%	2.51 [0.62, 10.21]
Dawood et al. 2020	56	60	55	60	2.8%	1.27 [0.32, 4.99]
Dawood et al. 2020 (1)	56	60	55	60	2.8%	1.27 [0.32, 4.99]
de Moraes et al. 2022	69	70	61	70	1.6%	10.18 [1.25, 82.68]
de Moraes et al. 2022 (1)	69	70	61	70	1.6%	10.18 [1.25, 82.68]
Han et al. 2015	29	30	29	30	1.0%	1.00 [0.06, 16.76]
Khunrang et al. 2021	170	200	175	200	5.3%	0.81 [0.46, 1.43]
Mohammadi et al. 2021	40	45	36	45	3.3%	2.00 [0.61, 6.52]
Mohammadi et al. 2021 (1)	41	45	36	45	3.1%	2.56 [0.73, 9.03]
Mohammadi et al. 2022	67	75	71	75	3.1%	0.47 [0.14, 1.64]
Mohammadi et al. 2022 (1)	67	75	72	75	2.8%	0.35 [0.09, 1.37]
Rodiriguez-Diaz et al. 2022	40	48	25	48	4.0%	4.60 [1.78, 11.86]
Rodiriguez-Diaz et al. 2022 (1)	36	48	25	48	4.3%	2.76 [1.16, 6.55]
Rodiriguez-Diaz et al. 2022 (2)	47	48	25	48	1.7%	43.24 [5.51, 339.28]
Santos et al. 2023	79	80	74	80	1.6%	6.41 [0.75, 54.48]
Santos et al. 2023 (1)	76	80	74	80	3.0%	1.54 [0.42, 5.68]
Saputra et al. 2016	60	60	58	60	0.9%	5.17 [0.24, 110.01]
Tan et al. 2019	84	90	83	90	3.5%	1.18 [0.38, 3.66]
Wu et al. 2021	87	90	88	90	2.0%	0.66 [0.11, 4.04]
Xia et al. 2018	164	180	169	180	4.5%	0.67 [0.30, 1.48]
Xia et al. 2018 (1)	168	180	169	180	4.4%	0.91 [0.39, 2.12]
Xia et al. 2018 (2)	161	180	169	180	4.6%	0.55 [0.25, 1.20]
Xia et al. 2020	164	180	169	180	4.5%	0.67 [0.30, 1.48]
Xia et al. 2020 (1)	167	180	169	180	4.4%	0.84 [0.36, 1.92]
Xia et al. 2020 (2)	161	180	169	180	4.6%	0.55 [0.25, 1.20]
Total (95% CI)		**3024**		**3024**	**100.0%**	**1.28 [0.94, 1.74]**
Total events	2783		2741			

Heterogeneity: $\text{Tau}^2 = 0.38$; $\text{Chi}^2 = 71.55$, df = 36 ($P = 0.0004$); $I^2 = 50\%$
Test for overall effect: $Z = 1.57$ ($P = 0.12$)

Fig. 6 Forest plot of randomized control trials on the effect of dietary probiotic administration on pre-challenge survival of tilapia (21 studies and 37 records)

survival rate in fish fed *Saccharomyces* and exposed to 40°C compared to fish that was not fed *Saccharomyces.* Also, the use of probiotics based on different feeding strategies may prove valuable. For example, Tachibana et al. (2020) tested the performance of probiotics based on four different feeding strategies. They concluded that, if given continuously, *Enterococcus faecium* can effectively protect fish, however, their growth and immune system were improved when administered over 14 days. This needs to be further researched to enrich existing information and possibly enhance the performance of probiotics.

According to Tachibana et al. (2020), the effectiveness of probiotics in providing long-term protection appears to be heavily influenced by factors such as the specific probiotic species, the concentration administered, and the duration of feeding. Considering the study of Aly et al. (2008b), it was observed that supplementing Nile tilapia's diet with *B. pumilus* at a concentration of 10^{12} CFU/g of the diet increased protection against *A. hydrophila* after one and two months of feeding, but not after eight months. However, a lower dietary concentration of 10^6CFU/g of diet did not provide any protection. Similarly, Aly et al. (2008a) found that supplementing the diet with *L. acidophilus*, *B. subtilis*, or a combination of both generally offered greater protection against *A. hydrophila*, *P. fluorescens*, and *S. iniae* after two months of feeding compared to one month.



A

Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Std. Mean Difference IV, Random, 95% CI
Abarike et al. 2018	146.75	9.01	100	63.33	3.48	100	4.1%	12.17 [10.93, 13.40]
Abu-Elala et al. 2013	154.97	5.01	78	136.24	4.12	78	4.2%	4.06 [3.51, 4.62]
Abu-Elala et al. 2013 (1)	174.68	2.55	78	136.24	4.12	78	4.1%	11.16 [9.87, 12.46]
Addo et al. 2017	675	92.9	240	620	98.3	240	4.3%	0.57 [0.39, 0.76]
Addo et al. 2017 (1)	598	78	240	620	98.3	240	4.3%	-0.25 [-0.43, -0.07]
Addo et al. 2017 (2)	591	85.3	240	620	98.3	240	4.3%	-0.31 [-0.49, -0.13]
Addo et al. 2017 (3)	622	118.3	240	620	98.3	240	4.3%	0.02 [-0.16, 0.20]
Aly et al. 2008	13.46	0.51	480	9.53	0.59	480	4.3%	7.12 [6.78, 7.46]
Aly et al. 2008 (1)	12.88	0.52	480	9.53	0.59	480	4.3%	6.02 [5.72, 6.32]
Aly et al. 2008 (2)	12.99	0.69	480	9.53	0.59	480	4.3%	5.39 [5.11, 5.66]
Bocamde et al. 2020	25.02	0.36	30	19.57	0.37	30	3.5%	14.74 [11.96, 17.51]
Cavalcante et al. 2020	13.46	0.51	40	9.53	0.59	40	4.1%	7.06 [5.85, 8.26]
Cavalcante et al. 2020 (1)	12.88	0.52	40	9.53	0.59	40	4.1%	5.97 [4.92, 7.01]
Cavalcante et al. 2020 (2)	12.99	0.59	40	9.53	0.59	40	4.1%	5.81 [4.79, 6.83]
Dowidar et al. 2018	1.75	0.17	60	1.13	0.1	60	4.2%	4.42 [3.75, 5.09]
Dowidar et al. 2018 (1)	1.87	0.12	60	1.13	0.1	60	4.2%	6.66 [5.73, 7.59]
Dowidar et al. 2018 (2)	1.74	0.07	60	1.13	0.1	60	4.2%	7.02 [6.05, 7.99]
El-Nobi et al. 2021	24.5	2.5	30	18.5	2.5	30	4.2%	2.37 [1.70, 3.04]
Han et al. 2015	139.99	8.34	30	113.53	6.56	30	4.2%	3.48 [2.66, 4.30]
Van doan et al. 2018	9.68	0.26	80	9.49	0.2	80	4.3%	0.82 [0.49, 1.14]
Van doan et al. 2018 (1)	10.93	0.33	80	9.49	0.2	80	4.2%	5.25 [4.59, 5.91]
Van doan et al. 2018 (2)	11.29	0.34	80	9.49	0.2	80	4.2%	6.42 [5.65, 7.20]
Van Doan et al. 2021	8.88	0.09	60	7.32	0.23	60	4.1%	8.88 [7.68, 10.07]
Zhu et al. 2019	90.83	5.05	150	45.83	1.91	150	4.2%	11.76 [10.78, 12.73]
Total (95% CI)			**3496**			**3496**	**100.0%**	**5.59 [4.36, 6.82]**

Heterogeneity: Tau² = 9.27; Chi² = 5859.63, df = 23 (P < 0.00001); I² = 100%
Test for overall effect: Z = 8.89 (P < 0.00001)

B

Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Std. Mean Difference IV, Random, 95% CI
Abomughaid 2020	1.9	0.02	30	1.4	0.01	30	5.1%	31.21 [25.41, 37.01]
Abomughaid 2020 (1)	1.9	0.02	30	1.4	0.01	30	5.1%	31.21 [25.41, 37.01]
Abu-Elala et al. 2013	1.88	0.04	78	1.52	0.04	78	8.2%	8.96 [7.90, 10.01]
Abu-Elala et al. 2013 (1)	2	0.05	78	1.52	0.04	78	8.1%	10.55 [9.32, 11.78]
Cavalcante et al. 2020	2.34	0.2	40	2.14	0.1	40	8.3%	1.25 [0.77, 1.73]
Cavalcante et al. 2020 (1)	2.22	0.2	40	2.14	0.1	40	8.3%	0.50 [0.06, 0.95]
Cavalcante et al. 2020 (2)	2.22	0.1	40	2.14	0.1	40	8.3%	0.79 [0.34, 1.25]
Dawood et al. 2020	2.71	0.02	60	2.21	0.06	60	8.0%	11.11 [9.64, 12.58]
Dawood et al. 2020 (1)	3.42	0.31	60	2.21	0.06	60	8.3%	5.38 [4.61, 6.16]
Van doan et al. 2018	2.53	0.08	80	2.21	0.08	80	8.3%	3.98 [3.44, 4.52]
Van doan et al. 2018 (1)	2.64	0.08	80	2.21	0.08	80	8.3%	5.35 [4.68, 6.02]
Van doan et al. 2018 (2)	2.7	0.07	80	2.21	0.08	80	8.3%	6.49 [5.70, 7.27]
Van Doan et al. 2021	3.23	0.02	60	2.48	0.04	60	7.1%	23.57 [20.51, 26.62]
Total (95% CI)			**756**			**756**	**100.0%**	**9.34 [7.23, 11.46]**

Heterogeneity: Tau² = 13.88; Chi² = 1169.77, df = 12 (P < 0.00001); I² = 99%
Test for overall effect: Z = 8.67 (P < 0.00001)

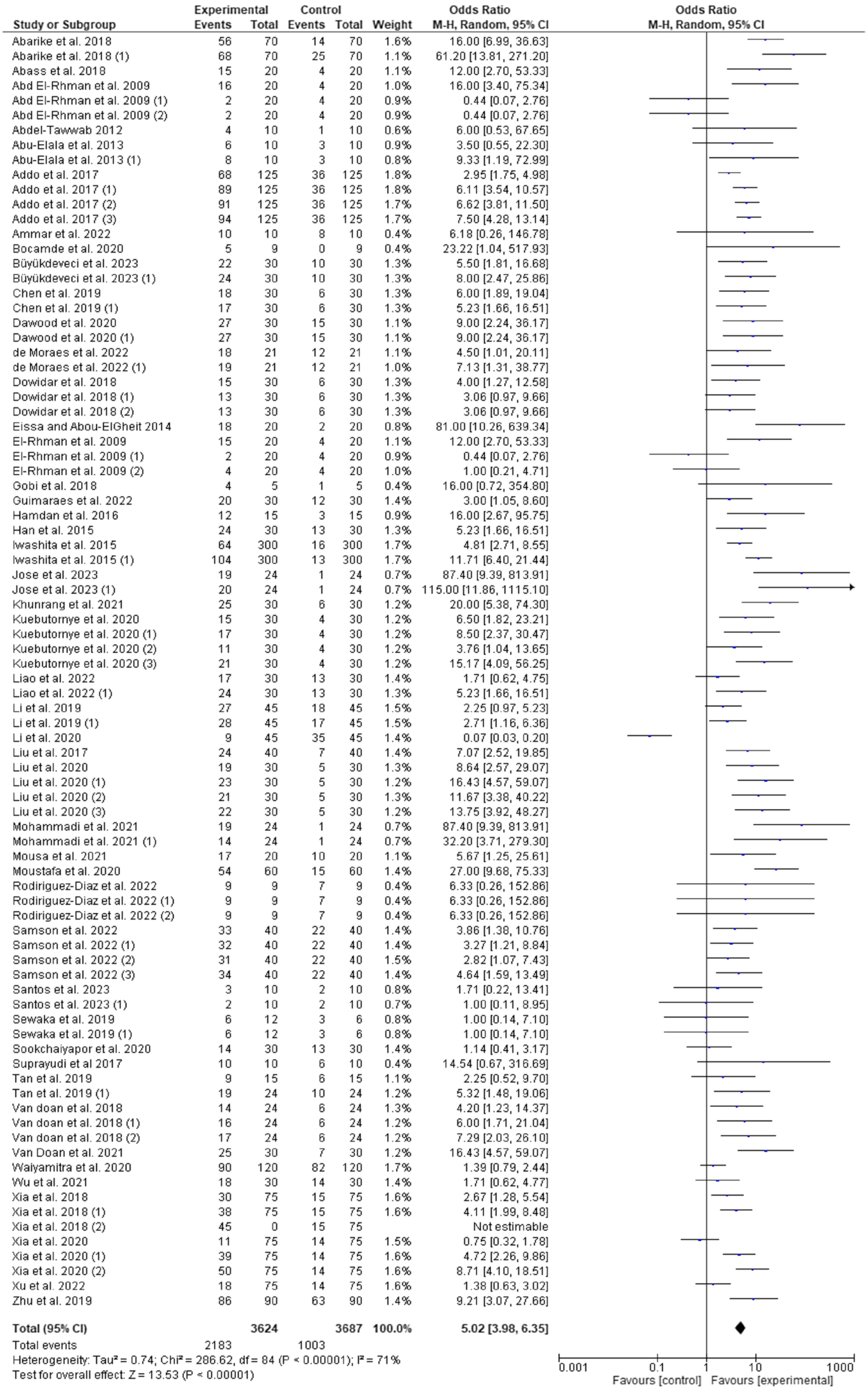
C

Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Std. Mean Difference IV, Random, 95% CI
Cavalcante et al. 2020	82.25	8.4	40	80.4	6	40	16.8%	0.25 [-0.19, 0.69]
Cavalcante et al. 2020 (1)	81.9	5	40	80.4	6	40	16.8%	0.27 [-0.17, 0.71]
Cavalcante et al. 2020 (2)	85.75	4.1	40	80.4	6	40	16.8%	1.03 [0.56, 1.50]
Dawood et al. 2020	40.22	0.72	60	37.32	0.72	60	16.6%	4.00 [3.38, 4.63]
Dawood et al. 2020 (1)	43.32	1.21	60	37.32	0.72	60	16.3%	5.99 [5.14, 6.84]
Suprayudi et al 2017	26.21	2.38	60	18.96	0.09	60	16.6%	4.28 [3.62, 4.93]
Total (95% CI)			**300**			**300**	**100.0%**	**2.61 [0.90, 4.33]**

Heterogeneity: Tau² = 4.51; Chi² = 298.99, df = 5 (P < 0.00001); I² = 98%
Test for overall effect: Z = 2.99 (P = 0.003)

Fig. 7 Forest plot of randomized control trials on the effect of dietary probiotic administration on (**A**) serum lysozyme (12 studies and 24 records), **B** phagocytotic index (6 studies and 13 records), and (**C**) phagocytotic activity of tilapia (3 studies and 6 records)

Defining the proportions of each bacterium in a multi-strain probiotic is a crucial parameter that has often been overlooked in previous journal articles. Many of these articles have applied equal proportions of each component without optimizing the proportions. It is also important to assess the safety hazards posed by these mixtures to fish hosts, consumers, and the environment, as well as their potential synergistic or antagonistic activities. This can be achieved through screening for specific virulence factors or antibiotic resistance genes, or through whole genome sequencing and analysis to gain a clearer understanding of the potential probiotic species (Melo-Bolívar et al. 2021). In addition, a meta-analysis of other pathogenic challenge routes including oral challenge (Panase et al. 2023), immersion, and intramuscular (Srisapoome and Areechon 2017), and alternative administration route of probiotics in culture water requires further



Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
Abarike et al. 2018	56	70	14	70	1.6%	16.00 [6.99, 36.63]
Abarike et al. 2018 (1)	68	70	25	70	1.1%	61.20 [13.81, 271.20]
Abass et al. 2018	15	20	4	20	1.1%	12.00 [2.70, 53.33]
Abd El-Rhman et al. 2009	16	20	4	20	1.0%	16.00 [3.40, 75.34]
Abd El-Rhman et al. 2009 (1)	2	20	4	20	0.9%	0.44 [0.07, 2.76]
Abd El-Rhman et al. 2009 (2)	2	20	4	20	0.9%	0.44 [0.07, 2.76]
Abdel-Tawwab 2012	4	10	1	10	0.6%	6.00 [0.53, 67.65]
Abu-Elala et al. 2013	6	10	3	10	0.9%	3.50 [0.55, 22.30]
Abu-Elala et al. 2013 (1)	8	10	3	10	0.8%	9.33 [1.19, 72.99]
Addo et al. 2017	68	125	36	125	1.8%	2.95 [1.75, 4.98]
Addo et al. 2017 (1)	89	125	36	125	1.8%	6.11 [3.54, 10.57]
Addo et al. 2017 (2)	91	125	36	125	1.7%	6.62 [3.81, 11.50]
Addo et al. 2017 (3)	94	125	36	125	1.7%	7.50 [4.28, 13.14]
Ammar et al. 2022	10	10	8	10	0.4%	6.18 [0.26, 146.78]
Bocamde et al. 2020	5	9	0	9	0.4%	23.22 [1.04, 517.93]
Büyükdeveci et al. 2023	22	30	10	30	1.3%	5.50 [1.81, 16.68]
Büyükdeveci et al. 2023 (1)	24	30	10	30	1.3%	8.00 [2.47, 25.86]
Chen et al. 2019	18	30	6	30	1.3%	6.00 [1.89, 19.04]
Chen et al. 2019 (1)	17	30	6	30	1.3%	5.23 [1.66, 16.51]
Dawood et al. 2020	27	30	15	30	1.1%	9.00 [2.24, 36.17]
Dawood et al. 2020 (1)	27	30	15	30	1.1%	9.00 [2.24, 36.17]
de Moraes et al. 2022	18	21	12	21	1.1%	4.50 [1.01, 20.11]
de Moraes et al. 2022 (1)	19	21	12	21	1.0%	7.13 [1.31, 38.77]
Dowidar et al. 2018	15	30	6	30	1.3%	4.00 [1.27, 12.58]
Dowidar et al. 2018 (1)	13	30	6	30	1.3%	3.06 [0.97, 9.66]
Dowidar et al. 2018 (2)	13	30	6	30	1.3%	3.06 [0.97, 9.66]
Eissa and Abou-ElGheit 2014	18	20	2	20	0.8%	81.00 [10.26, 639.34]
El-Rhman et al. 2009	15	20	4	20	1.1%	12.00 [2.70, 53.33]
El-Rhman et al. 2009 (1)	2	20	4	20	0.9%	0.44 [0.07, 2.76]
El-Rhman et al. 2009 (2)	4	20	4	20	1.0%	1.00 [0.21, 4.71]
Gobi et al. 2018	4	5	1	5	0.4%	16.00 [0.72, 354.80]
Guimaraes et al. 2022	20	30	12	30	1.4%	3.00 [1.05, 8.60]
Hamdan et al. 2016	12	15	3	15	0.9%	16.00 [2.67, 95.75]
Han et al. 2015	24	30	13	30	1.3%	5.23 [1.66, 16.51]
Iwashita et al. 2015	64	300	16	300	1.7%	4.81 [2.71, 8.55]
Iwashita et al. 2015 (1)	104	300	13	300	1.7%	11.71 [6.40, 21.44]
Jose et al. 2023	19	24	1	24	0.7%	87.40 [9.39, 813.91]
Jose et al. 2023 (1)	20	24	1	24	0.7%	115.00 [11.86, 1115.10]
Khunrang et al. 2021	25	30	6	30	1.2%	20.00 [5.38, 74.30]
Kuebutornye et al. 2020	15	30	4	30	1.2%	6.50 [1.82, 23.21]
Kuebutornye et al. 2020 (1)	17	30	4	30	1.2%	8.50 [2.37, 30.47]
Kuebutornye et al. 2020 (2)	11	30	4	30	1.2%	3.76 [1.04, 13.65]
Kuebutornye et al. 2020 (3)	21	30	4	30	1.2%	15.17 [4.09, 56.25]
Liao et al. 2022	17	30	13	30	1.4%	1.71 [0.62, 4.75]
Liao et al. 2022 (1)	24	30	13	30	1.3%	5.23 [1.66, 16.51]
Li et al. 2019	27	45	18	45	1.5%	2.25 [0.97, 5.23]
Li et al. 2019 (1)	28	45	17	45	1.5%	2.71 [1.16, 6.36]
Li et al. 2020	9	45	35	45	1.4%	0.07 [0.03, 0.20]
Liu et al. 2017	24	40	7	40	1.4%	7.07 [2.52, 19.85]
Liu et al. 2020	19	30	5	30	1.3%	8.64 [2.57, 29.07]
Liu et al. 2020 (1)	23	30	5	30	1.2%	16.43 [4.57, 59.07]
Liu et al. 2020 (2)	21	30	5	30	1.3%	11.67 [3.38, 40.22]
Liu et al. 2020 (3)	22	30	5	30	1.2%	13.75 [3.92, 48.27]
Mohammadi et al. 2021	19	24	1	24	0.7%	87.40 [9.39, 813.91]
Mohammadi et al. 2021 (1)	14	24	1	24	0.7%	32.20 [3.71, 279.30]
Mousa et al. 2021	17	20	10	20	1.1%	5.67 [1.25, 25.61]
Moustafa et al. 2020	54	60	15	60	1.4%	27.00 [9.68, 75.33]
Rodiriguez-Diaz et al. 2022	9	9	7	9	0.4%	6.33 [0.26, 152.86]
Rodiriguez-Diaz et al. 2022 (1)	9	9	7	9	0.4%	6.33 [0.26, 152.86]
Rodiriguez-Diaz et al. 2022 (2)	9	9	7	9	0.4%	6.33 [0.26, 152.86]
Samson et al. 2022	33	40	22	40	1.4%	3.86 [1.38, 10.76]
Samson et al. 2022 (1)	32	40	22	40	1.4%	3.27 [1.21, 8.84]
Samson et al. 2022 (2)	31	40	22	40	1.5%	2.82 [1.07, 7.43]
Samson et al. 2022 (3)	34	40	22	40	1.4%	4.64 [1.59, 13.49]
Santos et al. 2023	3	10	2	10	0.8%	1.71 [0.22, 13.41]
Santos et al. 2023 (1)	2	10	2	10	0.7%	1.00 [0.11, 8.95]
Sewaka et al. 2019	6	12	3	6	0.8%	1.00 [0.14, 7.10]
Sewaka et al. 2019 (1)	6	12	3	6	0.8%	1.00 [0.14, 7.10]
Sookchaiyapor et al. 2020	14	30	13	30	1.4%	1.14 [0.41, 3.17]
Suprayudi et al 2017	10	10	6	10	0.4%	14.54 [0.67, 316.69]
Tan et al. 2019	9	15	6	15	1.1%	2.25 [0.52, 9.70]
Tan et al. 2019 (1)	19	24	10	24	1.2%	5.32 [1.48, 19.06]
Van doan et al. 2018	14	24	6	24	1.3%	4.20 [1.23, 14.37]
Van doan et al. 2018 (1)	16	24	6	24	1.2%	6.00 [1.71, 21.04]
Van doan et al. 2018 (2)	17	24	6	24	1.2%	7.29 [2.03, 26.10]
Van Doan et al. 2021	25	30	7	30	1.2%	16.43 [4.57, 59.07]
Waiyamitra et al. 2020	90	120	82	120	1.7%	1.39 [0.79, 2.44]
Wu et al. 2021	18	30	14	30	1.4%	1.71 [0.62, 4.77]
Xia et al. 2018	30	75	15	75	1.6%	2.67 [1.28, 5.54]
Xia et al. 2018 (1)	38	75	15	75	1.6%	4.11 [1.99, 8.48]
Xia et al. 2018 (2)	45	0	15	75		Not estimable
Xia et al. 2020	11	75	14	75	1.5%	0.75 [0.32, 1.78]
Xia et al. 2020 (1)	39	75	14	75	1.6%	4.72 [2.26, 9.86]
Xia et al. 2020 (2)	50	75	14	75	1.6%	8.71 [4.10, 18.51]
Xu et al. 2022	18	75	14	75	1.6%	1.38 [0.63, 3.02]
Zhu et al. 2019	86	90	63	90	1.4%	9.21 [3.07, 27.66]
Total (95% CI)		**3624**		**3687**	**100.0%**	**5.02 [3.98, 6.35]**
Total events	2183		1003			

Heterogeneity: $\text{Tau}^2 = 0.74$; $\text{Chi}^2 = 286.62$, df = 84 ($P < 0.00001$); $I^2 = 71\%$

Test for overall effect: $Z = 13.53$ ($P < 0.00001$)

Fig. 8 Forest plot of randomized control trials on the effect of dietary probiotic administration on post-challenge survival of tilapia (48 studies, 85 records) investigation to confirm their positive impact on the growth and disease resistance. It is also important to keep in mind that most studies conducted in journal articles were on a laboratory scale, and it would be valuable to understand the scalability of the technology and the potential real-world benefits of fish farms. While laboratory experiments have shown that probiotics can enhance disease resistance in tilapia, it is crucial to validate these findings through extensive field trials. Field conditions may introduce various factors that can potentially influence the outcomes, making it necessary to gather empirical evidence in field settings (Srisapoome and Areechon 2017).

Despite the value of this meta-analysis, we identify that heterogeneity between studies could affect the overall conclusions of a meta-analysis. The studies included in the meta-analysis used different probiotic strains, dosages, or durations of supplementation, and this could lead to some measure of heterogeneity in the results, while the inclusion and exclusion criteria may also introduce some measure of bias. Similarly, differences in study design could also contribute to heterogeneity between studies. In addition, publication bias could also affect the results of meta-analysis because studies with negative results may be less likely to be published than those with positive results. As only English articles were included, a language bias is also possible. Notwithstanding these possible biases, the use of dietary probiotics can reduce the reliance on antibiotics and other chemical treatments, which can have negative impacts on fish health and the environment. Additionally, the improved growth performance and disease resistance of fish can lead to higher productivity and economic benefits for fish farmers.

Conclusion

This systematic review and meta-analysis provide strong evidence to support the use of dietary probiotics as a feed additive to improve the growth performance, immune response modulation, and disease resistance of tilapia pre- and post-challenge with common pathogenic pathogens. The results of this study suggest that the use of multi-strain probiotics and synbiotics can enhance the health and productivity of tilapia and may have greater applications in sustainable aquaculture production. However, further research is needed to explore the optimal dose, duration, and combination of probiotics and synbiotics, as well as the long-term effects on fish health and the environment.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10499-024-01404-8.

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Authors' contribution Abdulwakil Olawale Saba: Conceptualization, Methodology, Data curation, Writing- Original draft preparation. Ina Salwany Md Yasin: Conceptualization, Writing- Reviewing and Editing. Mohammad Noor Amal Azmai: Conceptualization, Methodology, Writing- Reviewing and Editing.

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Data availability Most of the associated data can be found in the manuscript. However, any other required data would be provided upon reasonable request from the corresponding author.

Declarations

Ethical approval This review and meta-analysis is based on publicly available information and requires no form of ethical approval.

Competing interests The authors declare the absence of any competing interests or personal connections that could potentially affect the results presented in the paper.

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