

REVIEW

Role of β -glucan on finfish and shellfish health and well-being: A systematic review and meta-analysis

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Abstract

This study aimed to conduct a systematic review and meta-analysis evaluating the inclusion of β -glucan in aquaculture animal diets and its impact on their health outcomes. Relevant studies were identified from Scopus and Web of Science databases. A total of 82 primary studies published between 1996 and 2024 were reviewed, of which 70 were included in the meta-analysis. The results revealed that the application of β -glucan to aquaculture animal's diets significantly enhanced specific growth rate (SGR; mean effect, $g = 2.71$; $p < 0.001$), feed conversion ratio (FCR; $g = -3.88$; $p < 0.0001$) and lowered mortality after exposure to pathogens. Likewise, β -glucan had a positive influence ($p < 0.0001$) on innate immune parameters (lysozyme and phagocyte activity, NBT, ACH50, and IgM). The study found that the effects of β -glucans varied among marine and freshwater fish where freshwater fishes ($g = 2.05$ – 6.57) exhibit better performance. This study also found a negative correlation between fish's innate immune response and trophic level, suggesting that fish with higher trophic levels may be less efficient at absorbing this bio-stimulant. Even though there were high heterogeneity ($I^2 = 73\%$ – 97% , $p < 0.05$) due to the diversity of tested organisms and publication bias, our model and findings are valid. The findings suggest that the dietary application of β -glucans can have beneficial effects on

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growth and immune responses especially for freshwater species. The validity of these observations needs to be confirmed by further prospective studies.

KEYWORDS

aquatic animal, growth performance, immune response, immunostimulant, meta-analysis, β -glucan

1 | INTRODUCTION

Due to evolving consumption patterns and population growth, aquaculture is projected to increase by 62% between 2010 and 2030, fulfilling over two-thirds of global fish and shellfish demand.^{1–5} Approximately 100 million individuals rely on aquaculture for livelihoods, indicating its substantial socio-economic impact.⁵ While some debates persist, aquaculture plays a pivotal role in addressing global food security and poverty reduction, aligning with the UN's 2030 Agenda for Sustainable Development Goals.⁶ Notably, besides serving as a vital protein and

income source, aquaculture offers ecosystem services like wastewater treatment and habitat restoration.^{7,8} However, sustainable aquaculture practices are imperative to ensure these benefits, as inadequate practices can strain water resources, exacerbate overfishing, introduce invasive species, and foster antimicrobial resistance.^{9,10}

Despite its significance, aquaculture faces numerous hurdles hindering its growth.¹¹ Aquatic animal diseases, exacerbated by global trade, system intensification, and climate change, pose a major obstacle.^{12,13} High-density cultures and intensified systems facilitate pathogen evolution and disease outbreaks, compelling farmers to resort to antibiotics and disinfectants.^{14,15} Nevertheless, the overuse of these chemicals compromises animal immune systems and fosters antibiotic-resistant bacteria, posing a global health hazard.^{16–18} Moreover, the use of antibiotics has caused serious problems, such as the aquatic environments and the negative impacts on the aquatic ecosystem, the persistence of antibiotic residues in fish meat, and its negative impacts on human health.^{19,20} In this context, alternative disease prevention strategies like functional feed supplements and vaccination have been proposed.^{21–23} While vaccination reduces antibiotic usage in certain sectors, its specificity and cost limit widespread application, especially for tropical diseases.^{24–26} Given the prevalence of multi-agent infections in aquaculture, a holistic approach is crucial. Functional feed supplements, including medicinal plants and probiotics, have gained traction for their potential to enhance fish performance, immune systems, and feed utilisation.^{21,27,28} Incorporating such supplements into fish diets could not only improve disease prevention but also contribute to better resource utilisation, fostering sustainable aquaculture development.²⁹

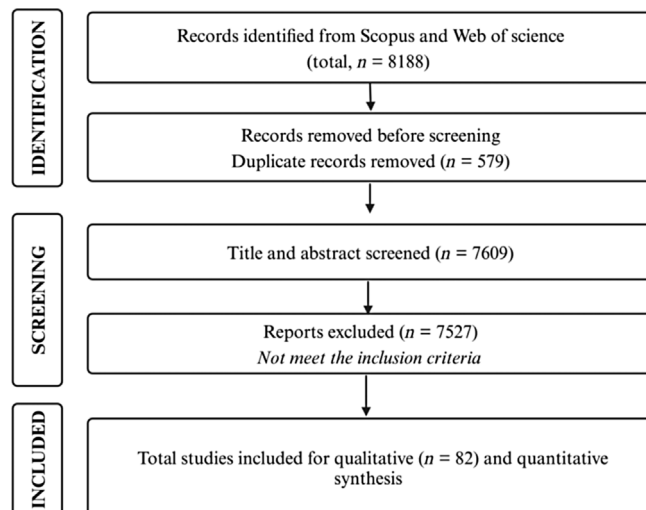


FIGURE 1 PRISMA flow chart for included studies.

TABLE 1 Summary of publication bias before and after removing outliers.

Variables	Heterogeneity ¹ (I ² , %)	Mean effect size ¹	Egger's regression		Study of outlier	Corrected effect size ²	Heterogeneity ² (I ² , %)
			Z	p-value			
SGR (n = 36)	99.63	2.91 [2.11 to 3.7]	8.27	<0.0001	21	2.71 [2.33 to 3.08]	94.60
FCR (n = 39)	99.95	−4.91 [−6.33 to −2.05]	−13.43	0.0001	22	−3.88 [−6.10 to −1.67]	99.85
Lysozyme activity (n = 46)	99.75	3.72 [2.47 to 4.99]	7.56	<0.0001	29	4.13 [3.67 to 4.59]	90.60
Phagocytic activity (n = 15)	99.73	4.37 [0.620 to 8.13]	9.02	<0.0001	8	2.39 [1.49 to 3.30]	97.33
ACH50 (n = 20)	98.44	1.7 [1.11 to 2.28]	1.65	0.0993	8	1.20 [0.95 to 1.46]	76.21
NBT (n = 21)	98.76	2.11 [1.11 to 3.12]	6.39	<0.0001	11	1.69 [1.31 to 2.07]	73.65
IgM (n = 10)	98.5	1.89 [1.06 to 2.73]	1.49	0.1387	5	2.32 [1.82 to 2.82]	82.02
Mortality (n = 8)	99.78	−5.85 [−7.94 to −3.75]	−12.39	<0.0001	2	−5.75 [−6.68 to −4.82]	96.33

β -Glucans have been used as potential prebiotics in aquaculture.³⁰ β -glucans comprise a group of β -D-glucose polysaccharides, naturally occurring in the cell walls of bacteria, fungi, and cereals, but with different properties dependent on the source.³¹ They form a linear backbone with 1–3 β -glycosidic bonds, but vary with respect to molecular mass, solubility, viscosity, branching structure, and gelation properties, causing diverse physiological effects in animals.³² In aquaculture, β -glucan is frequently added to feed to boost immunity, increase disease resistance, and enhance growth.^{33,34} By boosting the animal's resistance to infections and stimulating immunological cells, it can strengthen the host's immune response.^{35,36} β -glucan can protect aquatic animals against typical diseases like parasites, viruses, and bacterial infections.^{37,38} Furthermore, through increasing the number of helpful intestinal microbes, acidifying the digestive system, and lowering the amount of toxic intestinal metabolites, β -glucan contributes significantly to the improvement of the intestinal environment.^{39–41} Based on the literature, it is known that a β -glucan may have one or more modes of action, including the production of inhibitory compounds, enhancement of immune responses, improving water quality, competition for adhesion sites in the intestine, creation of proper interaction with phytoplankton, which is nutritionally important for fish and contains natural enzymes, helping digestive system of fish.^{33,42} However, the efficiency of β -glucans may change depending on breeding conditions, methods of consumption, dosage, and fish species.⁴³ Therefore, we are faced with a wealth of information on the therapeutic effect of β -glucans, which are sometimes contradictory. Meta-analysis is the use of specific statistical methods to summarise the results of independent studies to find the most accurate form of relationship between the variables.⁴⁴ Therefore, we conducted a systematic review and meta-analysis to investigate the effect of β -glucans on finfish and shellfish health and well-being.

2 | METHODS

2.1 | Literature search

The relevant literature search was conducted using the Scopus and Web of Science databases published from 1996 to 2024 and the combined keywords were used: (β -glucan*) AND (fish* OR crustacean*) AND (growth* OR immune*) AND (supplement* OR oral*). Article selection was based on the following criteria: (i) focused on aquatic animals; (ii) one of the following parameters was reported for both fish fed a β -glucan-free diet and fish fed with β -glucan-enriched diet: specific growth rate (SGR, %/day), feed conversion ratio (FCR), immunoglobulin (mg/mL), lysozyme activity (U/mL), phagocytic activity (%), complement activity (ACH₅₀, U/mL), respiratory burst activity (NBT, OD 630 nm), immunoglobulin M (IgM, mg/dL) or mortality after infected (%); (iii) mean, number of replicates and standard deviation or standard error were reported for each of the parameters; and (iv) effects of

TABLE 2 Summary of correlation between effect size and explanatory variables.

Variables	Lysozyme activity		Phagocytic activity		ACH50		NBT		IgM		Mortality		SGR		FCR							
	n	k	n	p	n	k	n	k	n	k	n	k	n	p	n	p						
β -Glucan	43	81	15	0.893	30	0.183	19	40	18	0.638	18	39	0.578	10	20	0.336	8	29	0.301	0.735	0.602	
Duration				0.006		0.135				0.004			0.004			0.262			0.262	0.461	0.865	
Trophic level				0.383		0.001				0.473			0.033			0.761			0.066	0.331	0.415	
Interaction ^a																						

Abbreviations: ACH₅₀, complement activity; IgM, immunoglobulin M; k, number of comparisons; n, number of studies; NBT, respiratory burst activity; p, p-value.

^aBetween duration and trophic level while both are significantly correlated with effect size.

β -glucan as feed supplement. Studies that evaluated the effect of mixed immunostimulants along with β -glucan at a time were not included. When the data were presented graphically, ImageJ (National Institutes of Health, Bethesda, Maryland, USA) was used to extract.

2.2 | Data analyses

A random-effects model was utilised to account for potential variability in effect sizes across studies. This is a standard approach to handle heterogeneity and is appropriate for meta-analyses where the true effect size may vary between studies.⁴⁵ Hedges' *g* effect size (*g*) was calculated as previously described.⁴⁶ The magnitude of standardised mean difference of *g* value was interpreted as follows: small effect = 0.2, medium effect = 0.5, and large effect = 0.8. A *g* value of zero indicated no significant difference in the tested

parameter with the administration of β -glucan. All statistical analyses were performed using the *metafor* package in R version 1.4.1103. The influence of explanatory variables (trophic level, experimental duration, β -glucan level) on Hedges' *g* effect size was performed with multiple linear regression under the ANCOVA framework.

2.3 | Heterogeneity and publication bias

Among-study heterogeneity was determined via I^2 index. Heterogeneity is considered high and low if I^2 index is $\leq 50\%$ and $>50\%$, respectively.⁴⁷ Egger's tests were used to assess publication bias. When publication bias was detected ($p < 0.05$) by Egger's test, we removed outliers and reported results from an outlier-free test according to the previous description.⁴⁸ Statistical significance was assumed with a *p*-value < 0.05 .

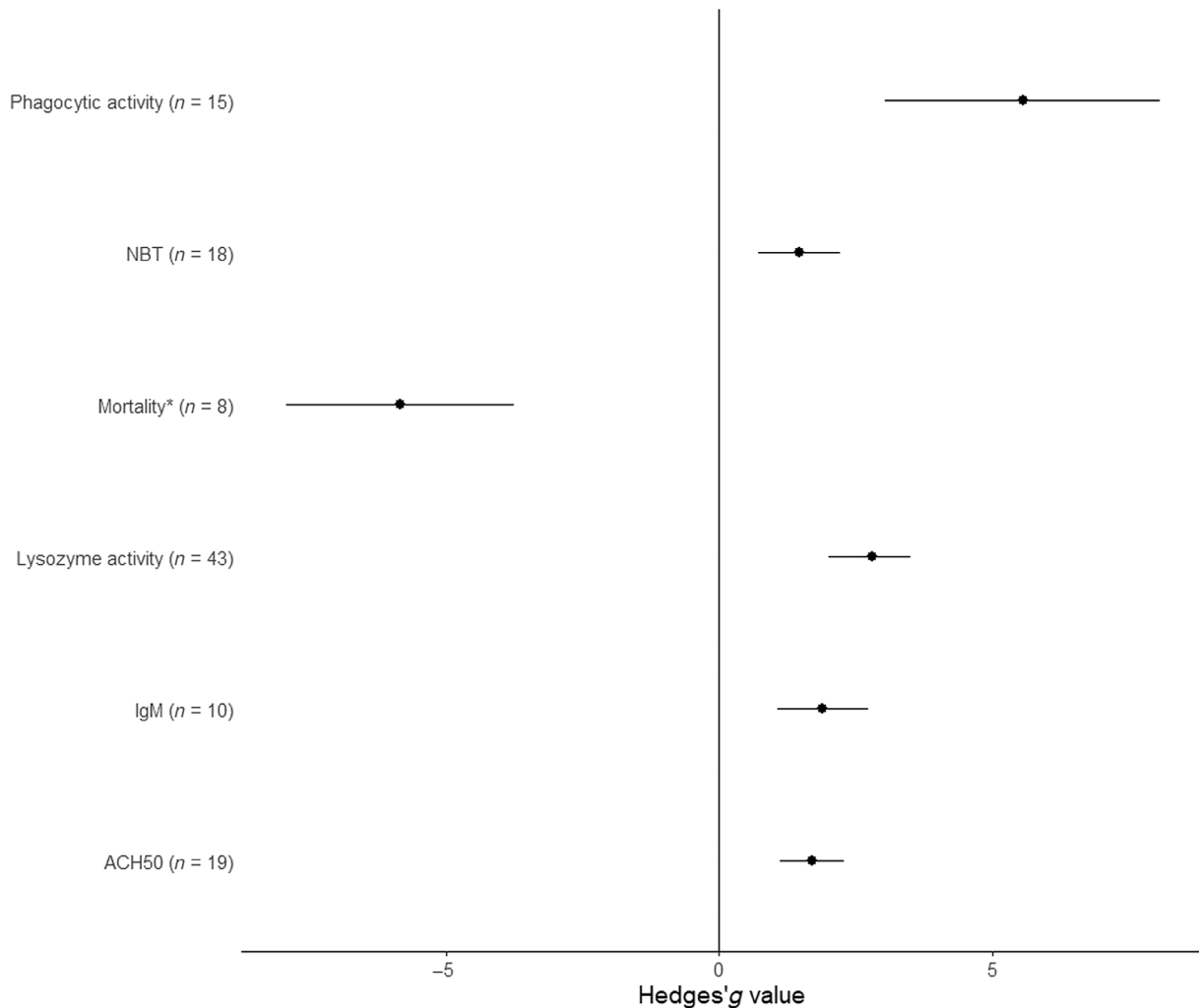


FIGURE 2 Forest plot for Hedge's effect size (mean and 95% confidential interval) of immune response indices. ACH50, complement activity; IgM, immunoglobulin M; NBT, respiratory burst activity.

3 | RESULTS

3.1 | Included studies and overview of dataset

We used the Scopus and Web of Science databases to collect relevant articles, resulting in 8188 records compiled into a single End-Note library. After removing duplicate articles, 7609 remained. Following careful screening of titles and abstracts, 7527 articles were excluded. Finally, 82 articles were selected for review, with 70 studies utilised in the meta-analysis. Please refer to Figure 1 for a visual representation of this process. From the 70 compiled studies investigating the effect of β -glucan on the immune response of fish and shrimp, a total of 33 fish species was investigated, in which two-thirds (70%) of all studies focused on freshwater fish (70% of the dataset), while the rest of 30% considered marine fish (Table S1). The trophic level (2.5–4.1, IR) and β -glucan levels (0%–0.1%) were found in the dataset. Most of β -glucan from the literature were extracted from yeast (*Saccharomyces cerevisiae*)

(93% of total studies) and other sources (such as *Laminarina digitata*, *Euglena gracilis*) (7%). β -Glucan was mainly obtained from commercial sources (94% of total studies), while laboratory extraction accounted for 6%. The administration duration of β -glucan on fed organisms ranged from 28 to 60 days (IR), with a mean value of 48.18 days (Table S1).

3.2 | Effect on β -glucan enrichment on growth production

Our findings indicated that the application of β -glucan on aquatic animal fish significantly enhanced growth performance (mean effect, $g = 2.71$; 95% CI = 2.33, 3.08; $p < 0.001$; $n = 36$). All β -glucan-fed organisms showed positive SGR compared to β -glucan-free addition (Table 1). Feed efficiency, as indicated by the FCR index, exhibited negative effects by supplementation of β -glucan (mean effect, $g = -3.88$; 95% CI = -6.10 , -1.67 ; $p < 0.001$; $n = 39$) (Table 1).

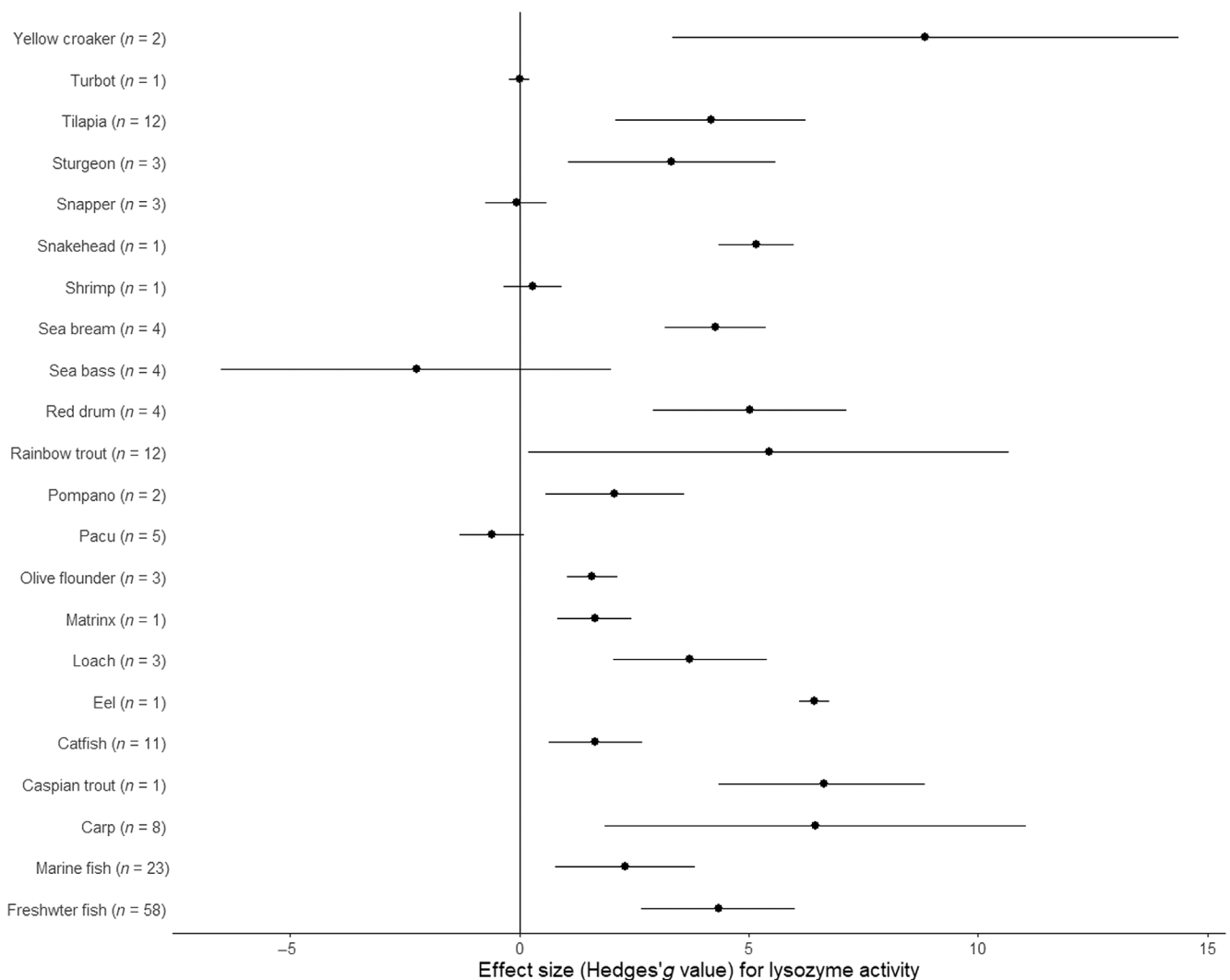


FIGURE 3 Hedge's effect size of lysozyme activity. 'n' indicates number of comparisons.

No significant association of explanatory variables was found for SGR and FCR (Table 2).

3.3 | Effects of β -glucan enrichment on immune parameters

The meta-analysis indicated that administration of β -glucan displayed positive results on Hedges' g effect size of lysozyme activity, phagocytic activity, ACH50, NBT, IgM ($p < 0.001$), while negative mortality was observed ($p < 0.001$), indicated better survival rate after exposed to the pathogen (Figure 2).

From 43 studies, the meta-analysis results confirmed the positive effect of β -glucan on lysozyme activities of aquatic animals as categorised in habitat groups, in which freshwater habitats (mean, 4.34) responded more significantly than marine ones did (2.30). Among fish species, carp, Caspian trout, and yellow croaker displayed the largest effect size of 6.45, 6.65, and 8.85, respectively, while snapper, turbot,

shrimp, sea bass, and pacu remained non-significant by multiple comparison results ($p = 0.848, 0.992, 0.300,$ and $0.056,$ respectively) (Figure 3).

The result of analysis from 15 publications showed a high phagocyte activity effect size of freshwater (6.57) compared to marine (1.85) habitats by β -glucan addition. Tilapia, pompano, flounder, and carp were positively affected by β -glucan supplement ($p < 0.001$) (Figure 4).

Most of fish species showed positive ACH50 effect size by administration of β -glucan, except for turbot ($p = 0.325$), tilapia ($p = 0.471$), and olive flounder ($p = 0.077$). Freshwater habitats also exhibited a larger effect size than did marine ones (Figure 5).

Supplementing β -glucan positively affected the effect size of NBT for carp ($p < 0.001$), pacu ($p < 0.0001$), sea bream ($p < 0.001$), shrimp ($p = 0.010$), tilapia ($p = 0.001$). In consistency with previous immune indicators, the effect size of NBT of marine and freshwater species is positive, while the latter was larger than the former (Figure 6).

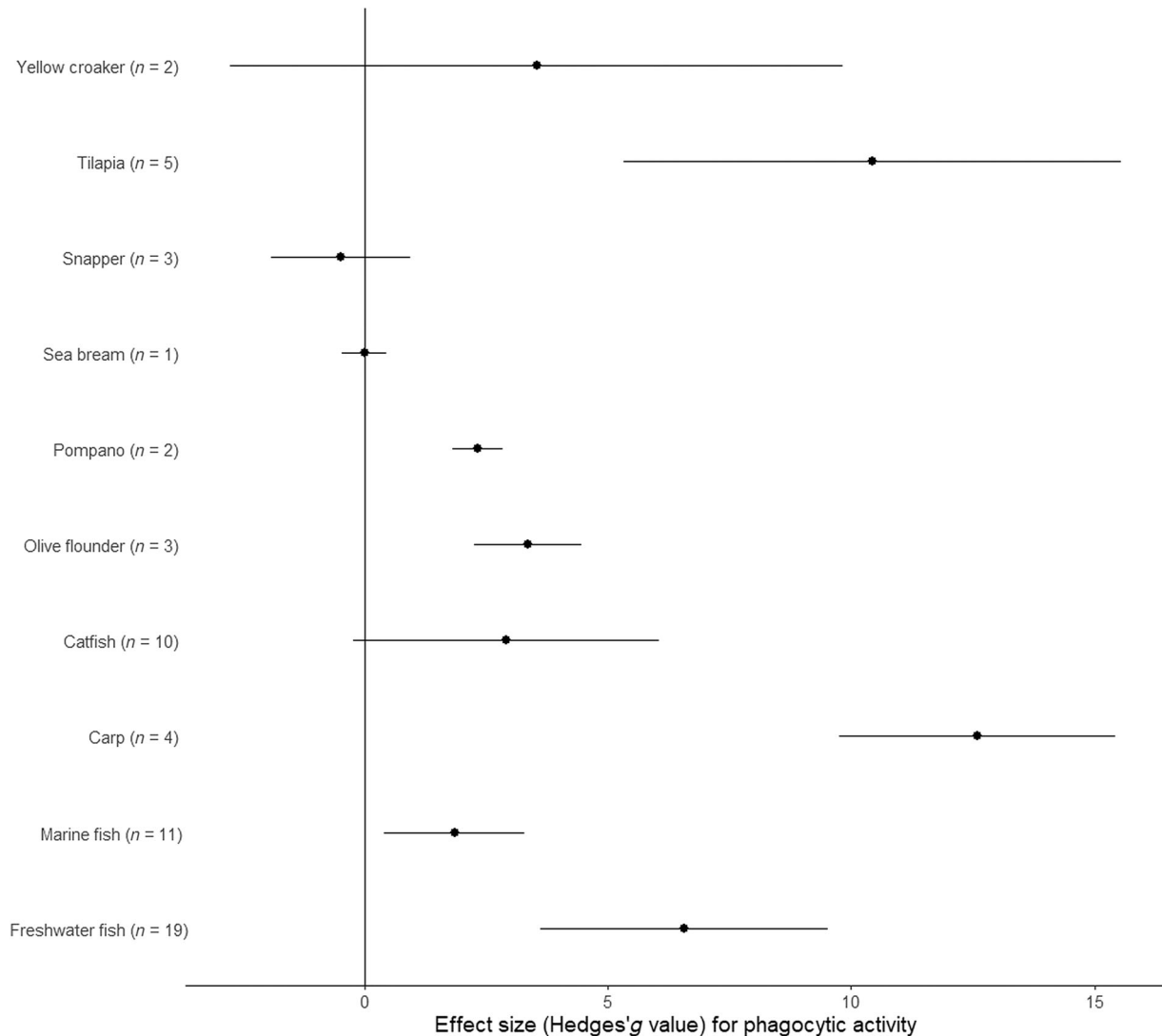


FIGURE 4 Hedge's effect size of phagocytic activity. 'n' indicates the number of comparisons.

Freshwater fish had a positive response of immunoglobulin M at the addition of β -glucan (2.05), except for sturgeon (-0.41 , $p = 0.060$), whereas marine fish showed an inverted response (-0.99) (Figure 7).

All species showed significantly lower mortality at the application of β -glucan after being infected with the pathogen in comparison with β -glucan-free diet (Figure 8).

3.4 | Meta-regression analysis

When considering the correlation between explanatory variables of β -glucan level, duration of the experiment, and trophic level with immune response effect size, there was no significant relationship between β -glucan level and effect size ($p > 0.05$). Experimental duration significantly correlated with lysozyme activity, ACH50, and NBT ($p < 0.05$), while trophic level had a significant relationship with phagocytic activity and NBT (Table 2, Figure 9).

3.5 | Heterogeneity and publication bias

The analysis indicated that all immune response indices had a remarkably high I^2 index (lysozyme activity, 99.37%; phagocytic activity, 99.73%; ACH50, 98.44%; NBT, 97.8%; IgM, 98.5%; and mortality after infected, 99.78%), suggesting high between-study heterogeneity.

The results from Begg's and Egger's tests showed no significant publication bias for ACH50 and IgM, while there was publication bias for the other four immune indices as indicated by the tests and asymmetrical funnel plots (Table 1, Figure 10).

4 | DISCUSSION

The role of β -glucans as an immunomodulatory additive on fish immunity has been extensively investigated in aquaculture nutrition research.^{35,38,49–51} Due to its ability to regulate cellular and humoral responses, restore cell homeostasis, and aid in the formation of

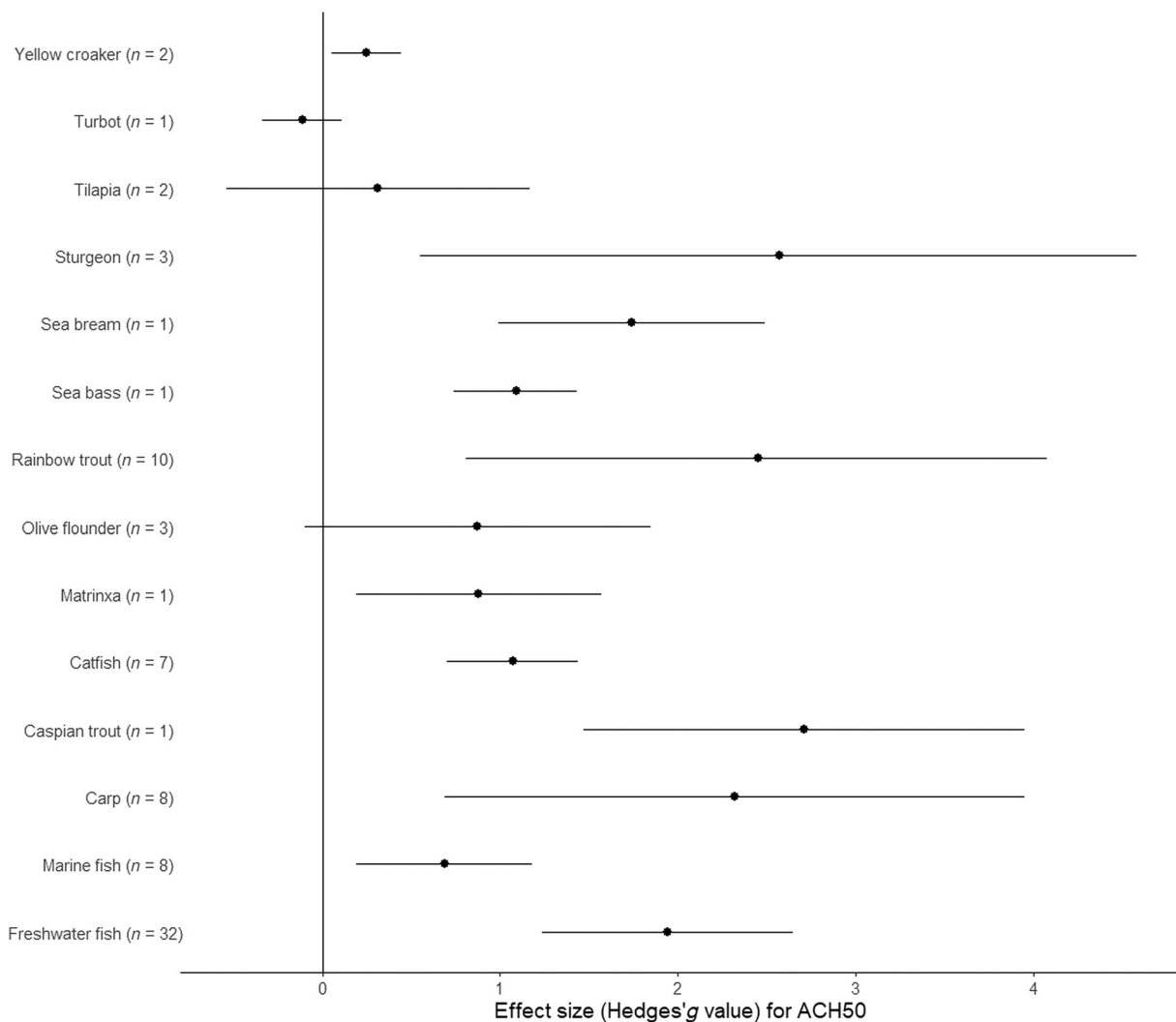


FIGURE 5 Hedge's effect size of complement activity (ACH50). 'n' indicates the number of comparisons.

immunity training, β -glucan has been widely utilised to boost the non-specific immune system.^{42,52,53} Pattern recognition receptors (PRRs) and β -glucans interact to initiate intracellular signalling that in turn activates humoral and cellular responses.^{54,55} Moreover, β -glucans can 'train' non-specific immunity by boosting the immune system and improving defence versus infections in the future.⁵⁶ Our meta-analysis provided a quantitative assessment of the effect of oral β -glucans administration on the immune response in aquatic animals. Overall, supplementing this immunostimulant improved growth performance and immune parameters while reducing FCR and mortality after exposure to various pathogens and ammonia stress. Although β -glucan significantly enhanced immune effect size in general, other explanatory variables—duration of the trial and trophic levels—were found to be significant covariances.

The high heterogeneity (>97%) across all immune parameters could be attributed to the diversity of tested organisms in the literature. Indeed, the compiled dataset covered 20 fish species from either marine or freshwater habitats. In the present study, publication bias was found in all investigated indices by Egger's tests (Table 1).

By removing strong outliers from the dataset, all effect sizes were still significant differences between β -glucans-supplemented and β -glucans-free applications (Table 1). Therefore, the findings from the present meta-analysis are valid even with publication bias.

Although the elimination of outlier studies reduced the heterogeneity of ACH50 and NBT, this value remained particularly high (>50%) across effect sizes. It is therefore apparent that the diversity of investigated fish in our study strongly affects between-study heterogeneity.

The growth and feed efficiency of aquatic animals at the supplementation of β -glucans fed were reported.^{57–60} β -glucan supplementation led to increased activity levels of important digestive enzymes and gut microbiota, which implies enhanced nutrient utilisation and digestion efficiency in aquatic farmed species.^{61–63} Supplementation of dietary β -glucan at concentrations ranging from 0.02% to 0.04% resulted in improved digestive capacity,⁶² antioxidant activity, and immune response of different freshwater fishes, river prawn, banana shrimp, and white shrimp.^{61,64–70} Adding β -glucans at concentrations between 0.4% and 0.6% to the diets of *Atractosteus tropicus* larvae

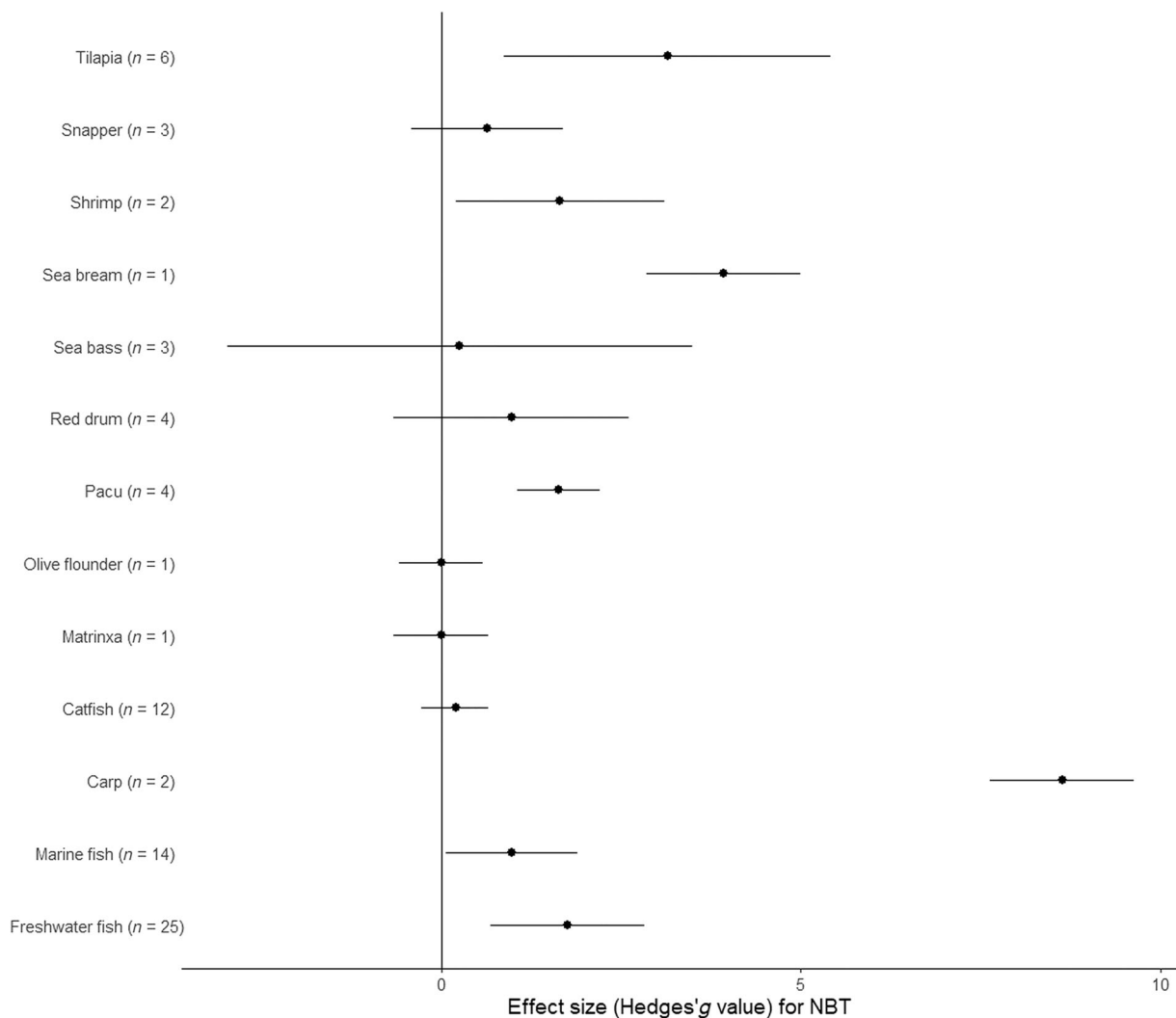


FIGURE 6 Hedge's effect size of respiratory burst activity (NBT). 'n' indicates the number of comparisons.

may enhance larval rearing.⁶¹ This is evidenced by the elevated activity levels of various digestive enzymes (such as lipase and trypsin) and the upregulation of immune system genes. Our findings indicate that the effect of β -glucans on innate immune indices varied widely among marine and freshwater fish. For example, Del Rio-Zaragoza et al.,⁷¹ and Ogier de Baulny et al.⁷² found no significant difference in lysozyme activities of turbot and snapper fed with enriched glucan relative to the control diet, while carp and trout showed a strong positive response to this supplementation.^{59,73,74} The phenomenon could likely be explained by the difference in the digestive tract system, nutritional requirement, and gut microbiota-driven immune response. Therefore, the use of β -glucan for some marine fish, such as sea bass, with the target of enhancing immune response should be carefully considered. The use of β -glucan in combination with other immunostimulants in marine fish could be an effective strategy.⁴⁹

Given that the immune response of aquatic animals is known to primarily rely on the duration of treatment, this relationship remains controversial in the literature. Douxfils et al.⁷⁵ reported a higher immune-related gene expression of rainbow trout fed β -glucan for

15 days than do 30 days. Similarly, lysozyme activity and ACH50 of tilapia were found to increase after 1 and 2 weeks of administrated β -glucans and to follow a slight decrease by the end of the 8-week experiment. A prolonged administration of β -glucan could lead to the immunity fatigue phenomenon found in fish.³⁸ In contrast, recent findings have developed a new term 'trained immunity' referring to the longer supplementation of β -glucan for enhancing the immune response in fish.⁷⁶ Our meta-regression models indicated that a relatively short feeding period (8 weeks for lysozyme activity and ACH50, and 3 weeks for NBT) was suitable for fish. This could hint at important information for further investigation at laboratory or commercial purposes to reduce treatment duration, thereby related costs. Moreover, our study found that all species exhibited significantly lower mortality rates when fed with β -glucan compared to those on a β -glucan-free diet after being infected with the pathogen. Some studies have also indicated that the dietary intake of β -glucan improved resistance to toxic stress (such as ammonia, fipronil, trichlorfon, and lead) to a certain extent, possibly by activating the antioxidative system in golden mahseer (*Tor putitora*), Nile tilapia, and African catfish (*Clarias gariepinus*).^{65,77,78}

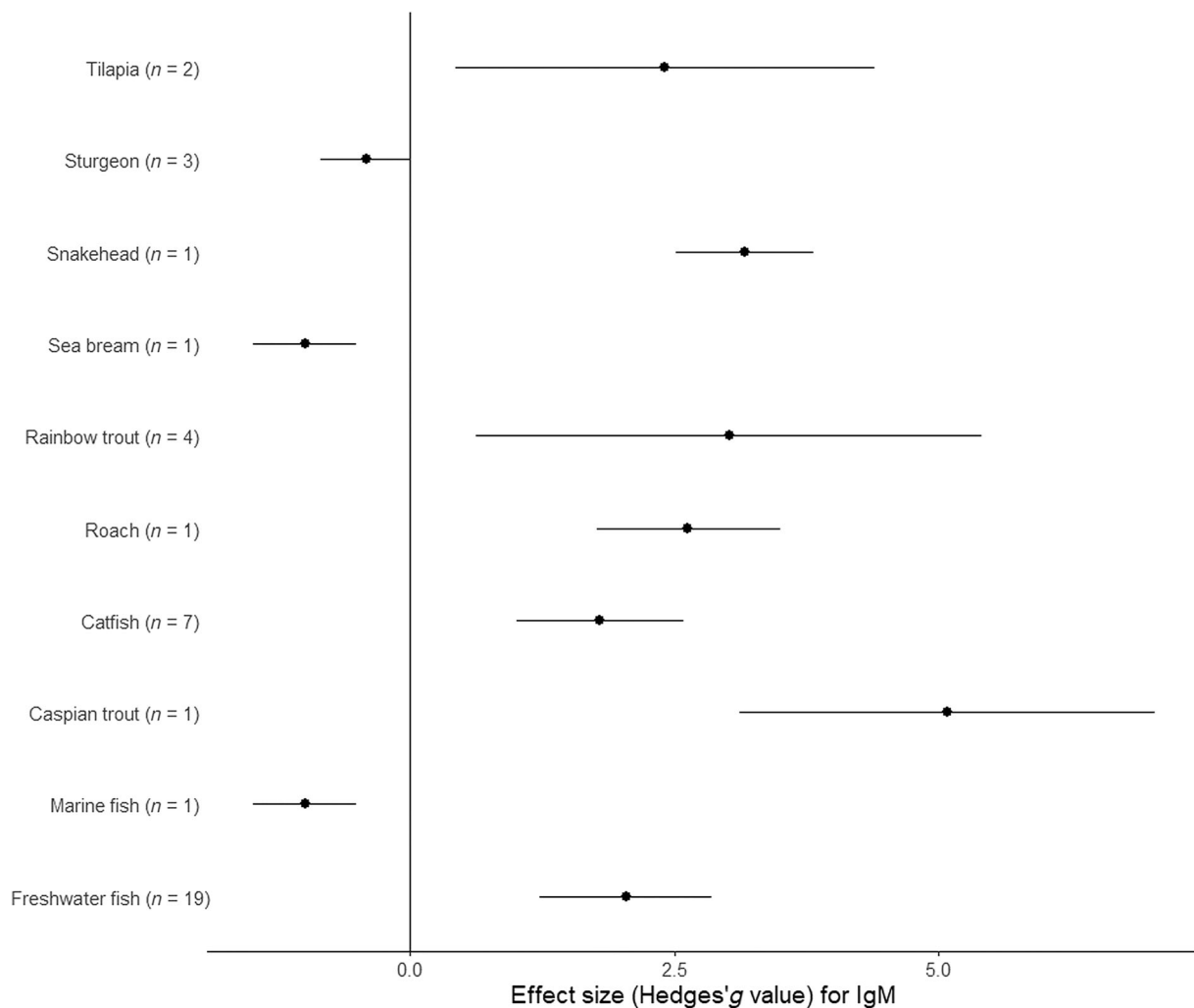


FIGURE 7 Hedge's effect size of immunoglobulin M (IgM) activity. 'n' indicates the number of comparisons.

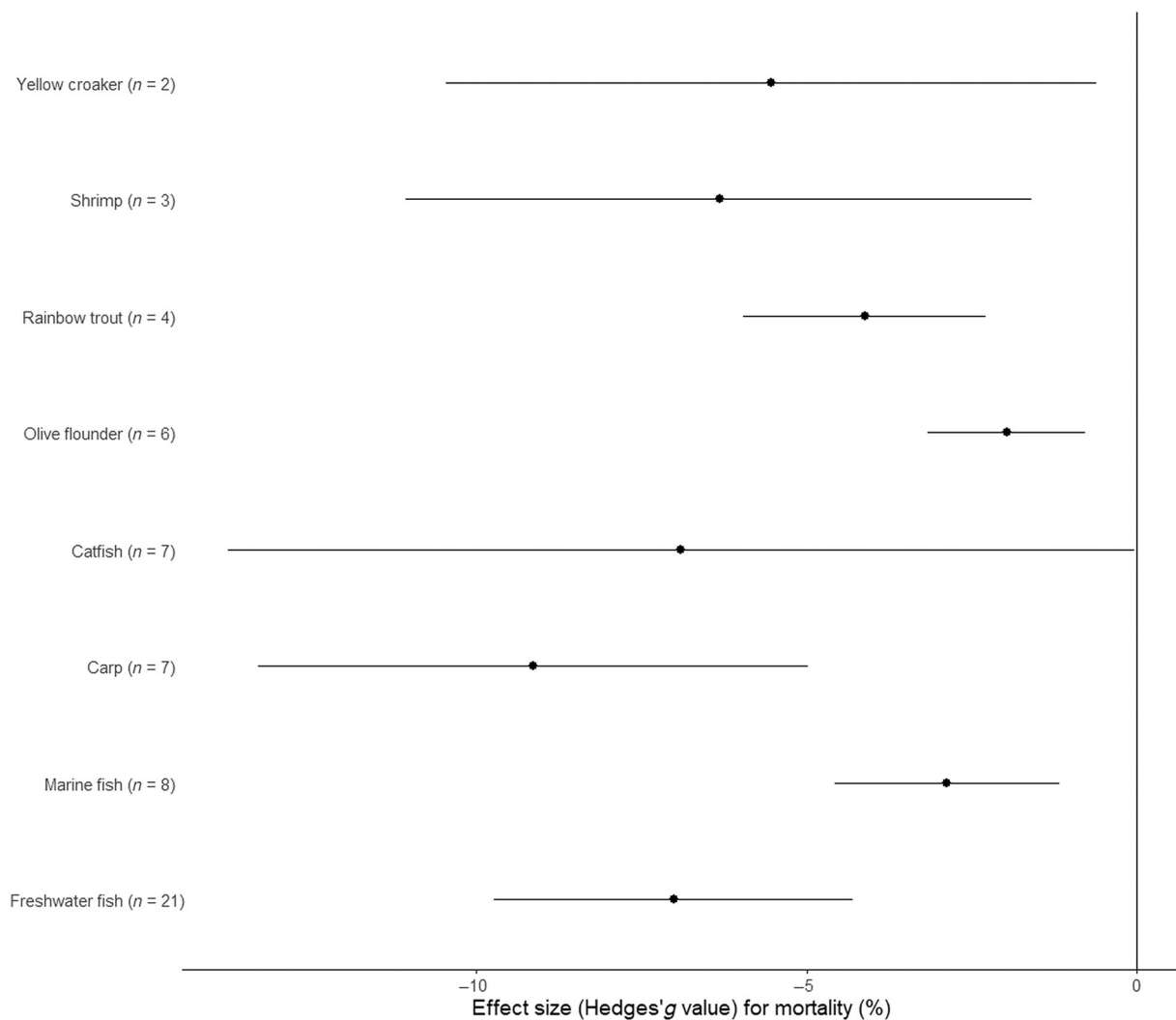


FIGURE 8 Hedge's effect size of mortality after exposure to pathogens. 'n' indicates the number of comparisons.

Recent studies have evidenced the influence of trophic levels on growth performance, immunity, and disease tolerance in fish.⁷⁹ The meta-regression analysis showed a negative correlation between the innate immune response of fish (phagocytic activity and NBT) and trophic level (Figure 8). The absorption of β -glucans has ultimately occurred in the intestine,³⁸ whose length has a negative relationship with trophic level. Therefore, the higher trophic level fish could be less effective in absorbing this bio-stimulant, thereby immune response.

5 | CONCLUDING REMARKS AND RESEARCH GAPS

The majority of the studies included in this review summarise that β -glucan supplementation improved the immunological properties (lysozyme activity, phagocyte activity, complement activity, respiratory burst activity, and immunoglobulin M) as well as reduced mortality among aquaculture species infected with the pathogen. It was found that a number of species, including carp, Caspian trout, yellow

croaker, tilapia, pompano, and flounder, responded strongly to the previously mentioned properties when provided with β -glucan supplement. Moreover, our findings concluded that results vary depending on fish species, environment, trophic level, and diet concentration. However, based on the results of this meta-analysis, adding β -glucan has a significant positive effect on the growth performance and immune responses of freshwater fish rather than marine fish. For β -glucan application, our meta-regression models suggest a short feeding period of 8 weeks for lysozyme activity and ACH50, and 3 weeks for NBT, which could provide valuable insights for further research to reduce treatment duration and associated costs. Moreover, there are limited studies examining the effects of adding dietary β -glucans on digestive enzyme activities in aquatic species.^{61,63} The findings indicate that β -glucans can modulate digestive enzyme activity in aquatic species, suggesting potential implications for nutrient absorption and overall digestive health in this species. Based on the fact that no information is available about the effect of immunostimulants on the 'good' gut microbiota with antagonistic activity against fish pathogenic bacteria, this should be a topic of further research, as

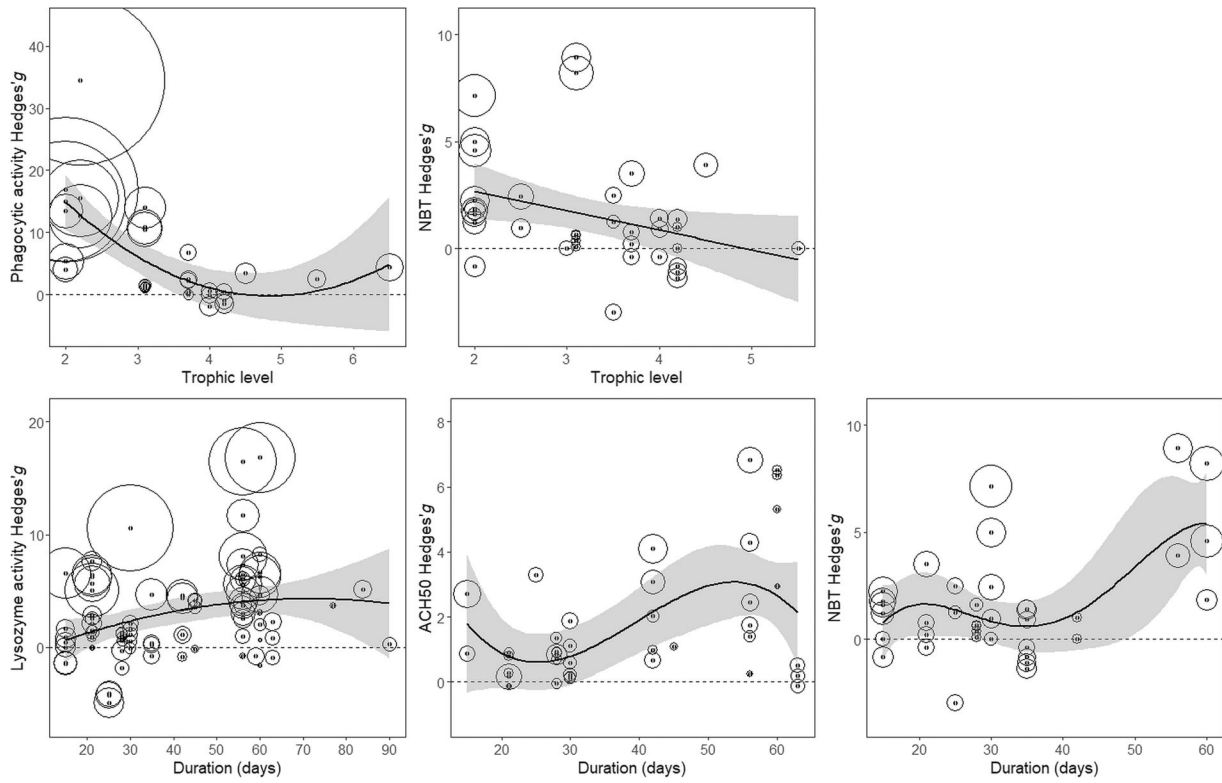


FIGURE 9 Significant correlation between explanatory variables and effect size. The centre and diameter of the circle represent the mean and the 95% confidence interval of effect size.

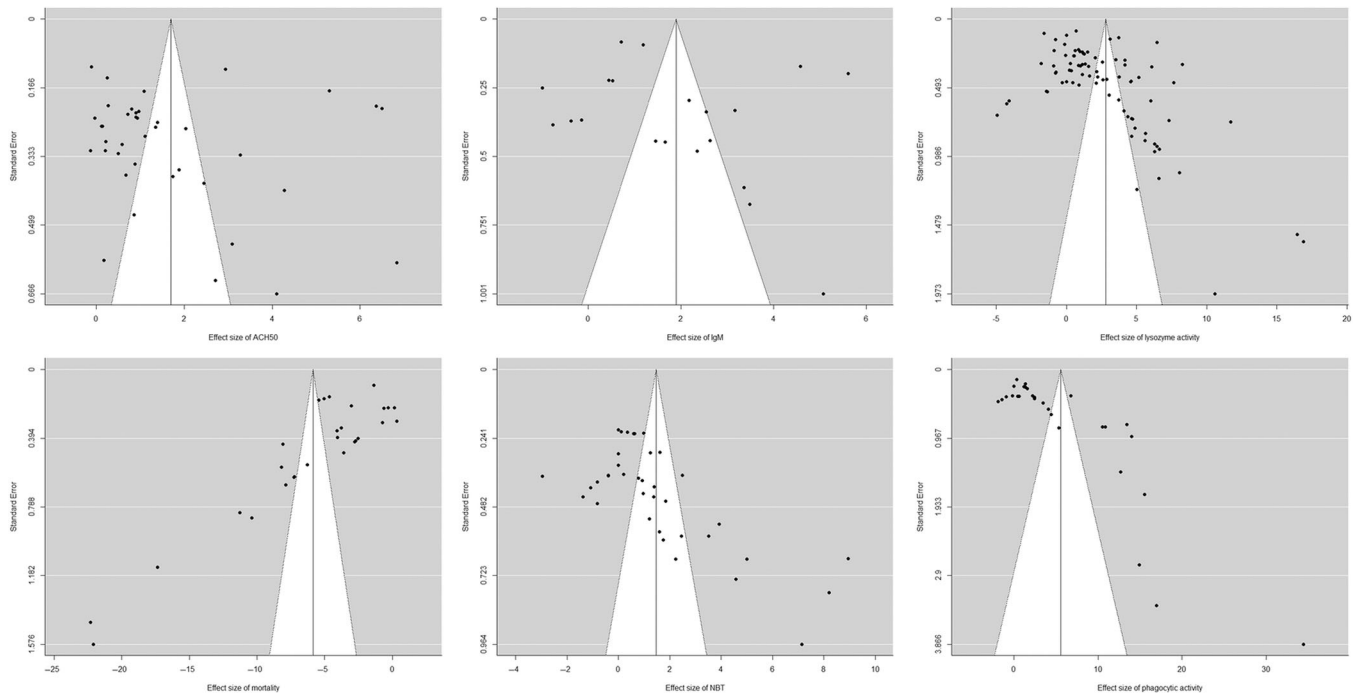


FIGURE 10 Funnel plots to assess the publication bias.

the gastrointestinal tract in fish is a potential port of entry for pathogenic bacteria.⁸⁰

AUTHOR CONTRIBUTIONS

Hien Van Doan: Conceptualization; investigation; funding acquisition; writing – original draft; validation; visualization; writing – review and editing; software; formal analysis; project administration; data curation; resources. **Md Afsar Ahmed Sumon:** Conceptualization; investigation; writing – original draft; methodology; validation; visualization; writing – review and editing; formal analysis; software; data curation; resources. **Hung Quang Tran:** Conceptualization; investigation; writing – original draft; methodology; validation; visualization; writing – review and editing; formal analysis; software; data curation; resources. **Chinh Xuan Le:** Conceptualization; investigation; writing – original draft; methodology; validation; visualization; writing – review and editing; software; formal analysis; data curation; resources. **Eman Y. Mohammady:** Conceptualization; investigation; writing – original draft; methodology; validation; visualization; writing – review and editing; formal analysis; software; data curation; resources. **Ehab R. El-Haroun:** Conceptualization; investigation; writing – original draft; visualization; writing – review and editing; formal analysis; supervision. **Seyed Hossein Hoseinifar:** Conceptualization; investigation; writing – original draft; methodology; validation; visualization; writing – review and editing; software; formal analysis; supervision. **Einar Ringo:** Conceptualization; investigation; writing – original draft; methodology; validation; visualization; writing – review and editing; software; project administration; supervision; data curation. **Stejskal Vlastimil:** Conceptualization; investigation; writing – original draft; methodology; validation; visualization; writing – review and editing; software; project administration; formal analysis; data curation; supervision. **Mahmoud A. O. Dawood:** Conceptualization; investigation; writing – original draft; writing – review and editing; visualization; project administration; supervision; data curation.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the authors up on request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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