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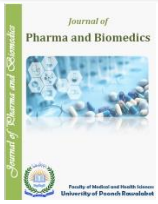

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Role of Vitamin D in Fish Growth and Immune Response: A Meta-Analysis

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A B S T R A C T

Vitamin D (VD) is a steroid hormone, of which vitamin D_3 [Cholecalciferol/ 25(OH)D_3] is the primary form found in fish. Fish mostly obtain this fat-soluble vitamin D3 from their diets, making it a common feed additive in aquaculture. This study performed a meta-analysis of published trials to determine the effects of Vitamin D3 on growth and immune response, providing a more reliable assessment of vitamin D effects across different fish species. A database was built from published literature regarding the addition of Vitamin D3 doses and their relation to the immune response and growth performance of fish. A literature search was carried out in Medline, Embase, Science Direct, Google Scholar, and The Scopus (up to 2024) for randomized controlled trials (RCTs) with studies selected based on criteria that included quantitative measures of growth and immunity. The recorded parameters were related to the immune response and Growth performance. The database contained a total of 65 data points from 41 studies that met the criteria. Effect sizes were calculated for the difference in the results of Vitamin D3 supplements and the control group. The pooled effect size was −0.024 (95% CI : −0.050, 0.003; 13 trials) of Vitamin D3 for Weight Gain, 0.026 (95% CI :-0.009, 0.060, 14 Trials) for SGR, -0.074 ((95% CI :- 0.113, - 0.035, 9 trials) for FCR , +3.41 (95% CI: 2.82 to 4.00, 10 trials) for Lysozyme activity and +14.17 (95% CI : 11.62 to 16.72, 10 trials) for SOD activity. We found that Vitamin D3 supplements significantly enhanced growth, immunity and disease survival of treated fish, regardless of the treatment duration, fish trophic level, and type of material used. Finally, we observed that studies need to improve the reporting of critical information about vitamin D_3 supplementation (e.g., source, dosage, formulation, and administration method), to enable better comparison across different experiments and enhance the repeatability of findings regarding its effects on fish growth and immune response.

Keywords: Vitamin D3, Fish, Aquaculture, Growth performance, Immune response, Randomized controlled trials.

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INTRODUCTION

The world population is increasing at a rate of 1.6% per year, which has put a great deal of pressure on the food industry (Sanò et al., 2019). The decline of wild fish and crustaceans production has made aquaculture an essential source of food for both people and animals (Alfiko et al., 2022). Fisheries and aquaculture are vital to global food security because they sustain the livelihoods and economies of many inland and coastal communities and offer a substantial source of protein to billions of people worldwide. Aquatic ecosystems are severely threatened by overfishing and environmental degradation, so sustainable management is required to ensure that future generations can continue to benefit from these resources. Almost 3 billion people worldwide rely on fish for 20% of their animal protein (Singh et al., 2022). Sustainable fisheries management contributes to increased food security by preserving a steady supply and controlling fish prices (Poczta-Wajda, 2024).

Around 178 million tons of aquatic animals were produced worldwide in 2020; by 2030, that amount is expected to rise to 202 million tons. About 16 million tons were used to produce fishmeal and oil, while 20 million tons were used for non-human food purposes. Nearly 157 million tons (89%) of these were intended for human consumption. In 2020, fishing and aquaculture generated an estimated 367 billion euros, of which 239 billion came from. Over 3 billion people worldwide rely on fish as their primary source of protein (Boyd *et al.,* 2022).

Cholecalciferol, another name for vitamin D3, is a naturally occurring form of vitamin D that is produced in the skin when it is exposed to ultraviolet B rays. It comes in a variety of formulations, such as oral tablets, softgels, liquid drops, injectable forms, topical creams, and fortified foods. It can be found in supplements and fortified foods. According to Khan et al. (2022), vitamin D3 is essential for immune system regulation, muscle function maintenance, cardiovascular health management, rickets prevention, bone mineralization, improved calcium and phosphate absorption, and overall health maintenance. It has been connected to seasonal affective disorder, depression, and cognitive decline. It is also being studied for cancer prevention. The formulation of vitamin D3 is intended to improve bioavailability, stability, and patient compliance. It is crucial for immune system function, bone development, and general health maintenance (Stasiak et al., 2019).

Fish is a rich source of protein, minerals, and omega-3 fatty acids, which are beneficial for cardiovascular health, brain development, and anti-inflammatory properties(Tahergorabi & Hosseini, 2018). Consuming fish regularly reduces the risk of chronic diseases and maintains adequate levels of Vitamin D, which is essential for overall health. Fish's higher bioavailability of these nutrients makes it a potential treatment for nutrient deficiencies, and its micronutrients promote bone health and cognitive function(Prakash *et al*., 2023).

Vitamin D facilitates calcium absorption, which is necessary for bone growth and mineralization. A systematic analysis and meta-analyses revealed links between vitamin D insufficiency and a number of illnesses .The maintenance of serum calcium and phosphate levels depends on vitamin D. Its significance in health is further demonstrated by the fact that it helps to prevent respiratory infections, chronic diseases, and boosts immunological function in general (Liu et al., 2020).

Vitamin D is referred to as a secosteroid due to its broken steroid ring structure. The binding of 1α,25-dihydroxyvitamin D3 to the VDR requires the presence of hydroxyl groups at positions 1 and 25(Wanat et al. , 2018). The A-ring of vitamin D can adopt different conformations (α and β) due to hydrogen bonding interactions, which changes how well it binds to receptors (Wanat et al., 2018). By binding to VDR and causing conformational changes that facilitate its interaction with the retinoid X receptor (RXR), 1,25(OH)2D3 forms a heterodimer that regulates gene transcription (Belorusova & Rochel, 2024). Numerous vitamin D analogs have been found through structural investigations to alter this interaction, indicating the possibility of medicinal uses (Fabisiak *et al*., 2024).

In a more focused research, scientists examined European sea bass to determine how vitamin D3 in the diet affects the immune system. According to their findings, giving vitamin D3 for two or four weeks significantly improved a number of immune markers, including the activation of genes linked to the immune system and modifications to the structure of gut tissue. In a similar vein, a high-dose vitamin D3 diet decreased Nocardia seriolae infections in largemouth bass by strengthening the fish's immune responses, specifically phagocytic activity in head kidney macrophages (Zhao et al., 2024).

Wang et al. (2024) found that vitamin D regulates the intestinal mucosal barrier, affecting gut microbiome composition, immune cell differentiation, and immune factors. Vitamin D deficiency increases intestinal illnesses and disrupts gut flora balance. Cholecalciferol, produced by converting lanolin into 7-dehydrocholesterol, is produced industrially through wool scouring, extraction, purification, and UV conversion into supplements (Wu *et al.,* 2020).

Fish process vitamin D through similar biochemical pathways as terrestrial vertebrates, with sites and regulation varying by species. Fish skin can convert 7-dehydrocholesterol to vitamin D, and the liver and kidney play key roles in hydroxylation. Fish have a parathyroid hormone-related protein (PTHrP) to control vitamin D metabolism. Fish maintain calcium-phosphorus balance in diverse aquatic environments through efficient transport and degradation of vitamin D. Fish can create active vitamin D3 by hydroxylating these forms in the liver and kidney. Cultivated fish rely on commercial diets, with VD3 being more accessible in aquatic feed. Fish store vitamin D mostly in D3 form, but the ratio of D2 to D3 in plankton does not represent this. Vitamin D supplementation can enhance growth and feed efficiency in various fish species, including tilapia and black carp. It plays a crucial role in enhancing the immune system of fish, particularly through its effects on mucosal barriers, gut microbiota, and direct immune responses (Knuth *et al.,* 2020).

A meta-analysis was conducted to fill knowledge gaps, support evidence-based aquaculture nutrition practices, and define the effects of vitamin D3 on fish growth and immune response. This research addresses a gap in comprehensive meta-analytical studies on vitamin D3 in fish, aligning with the author's interest in fish physiology, nutrition, and data-driven research methodologies. The meta-analysis produced a more reliable estimate of vitamin D's effects on fish growth and immune response, guiding future research priorities and improving estimates of effect sizes.

The increasing demand for fish protein worldwide requires sustainable aquaculture practices to achieve optimal fish health and growth. Understanding the role of Vitamin D in fish development and immunity is therefore necessary for improving aquaculture productivity. The aim of this meta-analysis is to evaluate and compile research on the effects of vitamin D supplementation on fish growth parameters and immune response in various fish species. In particular, the study looks at, "What is the impact of dietary Vitamin D_3 supplementation on growth performance and immune parameters in fish?"

RESEARCH METHODOLOGY

2.1 Study Design

A meta-analysis of peer-reviewed experimental studies on the impact of dietary vitamin D supplementation on fish growth performance and immune response was conducted. The study found that vitamin D supplementation significantly improved growth parameters and immune indicators in various fish species. The study followed the PRISMA 2020 Statement guidelines, ensuring transparency, reproducibility, and completeness in evidence synthesis, offering insights for aquaculture nutrition practices and future research directions.

2.2 Literature Search Strategy

A comprehensive search for journal articles or Development of the database was conducted in major bibliographic databases—Scopus, PubMed, Web of Science, SciHub and Google Scholar—covering all records up to 31 December 2024 for randomized controlled trials of dietary vitamin D3 supplements. Search terms or keywords that were used "vitamin D3" OR "1α,25-dihydroxyvitamin " OR calcitriol, fish OR "fish species", growth OR "feed conversion ratio" OR "weight gain OR immunity OR "immune response" OR lysozyme OR "Cytokines level", ―Cholecalciferol and ―Fishes.

2.3 Inclusion and Exclusion Criteria

Inclusion Criteria

The following inclusion criteria were used to select studies in order to assure their relevance and consistency. Peer-reviewed experimental studies that evaluate vitamin D_3 supplementation in fish diets, Research type including in vivo experiments. Every species of marine and freshwater finfish. Adding different amounts of vitamin D_3 to the diet or comparing it to a control diet for intervention. Control groups that were given a normal baseline diet devoid of vitamin D_3 or no supplements. For Measured results it is necessary to report at least one of the following parameters: Growth performance metrics (feed conversion ratio, weight increase, and specific growth rate) and Immune response markers (Sodium Oxide Desmutase Activity and lysozyme activity). The study provides sufficient information to calculate effect sizes (e.g., means, standard deviations, sample sizes). Searches were limited to English-language publications. The data were recorded between upto 2024. Random-effects models were used to determine the pooled effect sizes.

Exclusion Criteria

Excluded were in vitro/cell-culture studies, non-fish species or research on aquatic animals other than fish, such as mollusks and crabs., reviews, duplicate reports of the same data, Reviews, opinions, case reports and studies without controls or quantitative data. Selection is summarized in a PRISMA flow diagram Figure. Duplicate records were removed in EndNote X9.

2.4 Study Selection and Screening process

After searching using the keywords above 63 articles were found. 11 dupplicate articles were removed before screening then next stage was an abstract and title evaluation, which resulted in 44 articles that could potentially be used after excluding 8 articles based on title and adstracts . The next process was an evaluation of the entire article , 6articles excluded due to non-fish species , 5 articles unrelated to vitamin D3 were excluded, 4 article not focused on growth and immunity, so that was removed, which resulted in 29 articles that could be used. The number of studies thus selected for further analysis varied depending on each parameters. Rigorous screening of the studies included in the primary data set was performed to select only those studies appropriate for further meta-analytic procedure. Only a limited number of criteria were compared in this meta-analysis due to the lack of sufficient data for many other response criteria.Figure1 shows a PRISMA 2020 flow diagram of the research selection process.

2.5 Data Extraction

The basic information about each eligible study was extracted including Study characteristics (first author, publication year, country), fish species, Fish initial weight, stocking density and study duration. Finally, the Generalized data from 29 journal articles were entered into the database Table 3.1.

2.6 Quality Assessment / Risk of Bias

The study assessed the quality of papers and reports using criteria like randomization, statistical analysis, and treatment comparability. Non-peer-reviewed studies were included in the meta-analysis only if they met selection criteria. Funnel Plots are representing the Quality assessment of each parameter.

2.7 Effect Size Calculation

Meta-analysis was applied to quantify the effect of Vitamin D3 in diets on fish growth. The Cochrane Review Manager (Cochrane RevMan Version 5.3) was used to pool and analyse results from the individual studies reviewed. Pooled results were reported as mean differences with 95% CI and with two-sided p-values. In order to minimize potential bias resulting from methodological differences between research, a random effects model that accounts for inter- study variation was applied.

Combined standard error

$$SE_{\Delta} = \sqrt{SE^2_{\text{ctrl}} + SE^2_{\text{exp}}}.$$

$$T = \frac{\Delta}{SE_{\Delta}},$$

$$\Delta = \bar{X}_{\text{exp}} - \bar{X}_{\text{ctrl}}$$

2.8 Hypothesis Testing

To assess the effect of dietary vitamin D_3 supplementation on fish growth performance and immunological response, standardized effect sizes were calculated for each included study. The primary statistic used was the Standardized Mean Difference (SMD), particularly Hedges' g, which corrects for small sample size bias (Hedges & Olkin, 1985). This metric makes it possible to compare research using various scales or measuring units.For each study, the following data were extracted: Mean value of the outcome (e.g., weight gain, lysozyme activity) for both the control and vitamin D_3-supplemented groups, Standard deviation (SD) or standard error (SE) of each group, Sample size of each group.

Effect size by Hedges' *d:*

$$d = \frac{\bar{X}_1 - \bar{X}_2}{S_p}$$

Where:

- $\bar{X}_1$ = Mean of the treatment group
- $\bar{X}_2$ = Mean of the control group
- S_p = **Pooled standard deviation**

$CI = MD \pm 1.96 \times SE_{MD}$

CI is Confidence Interval, the range of values within which the true population parameter is likely to fall. **MD is** Mean Difference, the difference between the means of two groups.± **1.96 × SE** is part of the formula adds and subtracts the margin of error (1.96 times the standard error) to the mean difference to create the upper and lower bounds of the confidence interval. **1.96 is** Z-score for a 95% confidence level for a normal distribution.

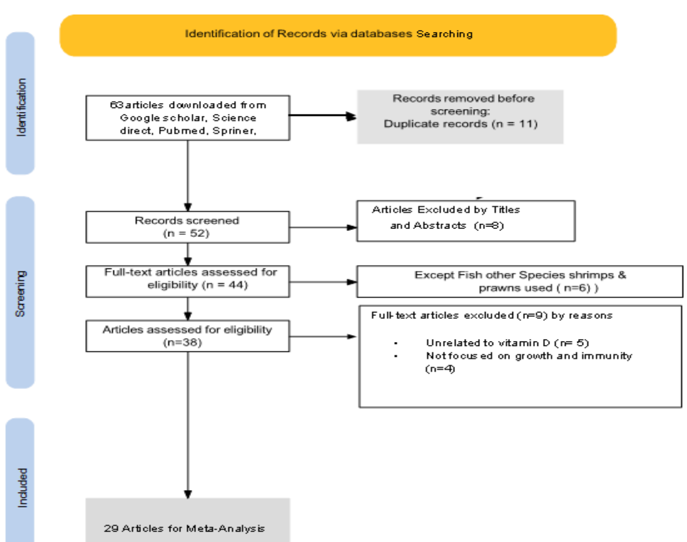

Figure 1.The flow chart for detailed steps of literature search.

Table 1. Summary of Selected Studies for Meta-Analysis,with respect to different response criteria tested on basis of Vitamin D3

Author/ Year	Region/study location	Specie	Sample size	Duration	Fish initial weight (g) mean ± SD
(Lin et al., 2022)	Kaohsiung, Taiwan	Zebrafish (*Danio rerio)*	20	30	NM
(Shao et al., 2022)	handong Province, China	Turbots *Scophthalmus maximus*	60	560	13 ± 0·08
(Wang et al., 2024)	Sichuan Province, China	Grass carp (Ctenopharyngodon idella)	540	560	3.05±0.03
(Sun et al., 2024)	Chengdu, China	Grass carp (Ctenopharyngodon idella)	540	70	19.70 ± 0.20
(Cheng et al., n.d.)	Hangzhou, China	Catfish (Pelteobagrus fulvidraco)	36	84	22.34 ± 1.8
(Inayat et al., 2020)	Pakistan	*Labeo rohita*	225	90	277.64 ± 2.9
(Horvli, 1997)	Norway	Atlantic salmon (*Salmo salar)*	600	77	177± 0.2
(GRAFF, HØIE, TOTLAND, & LIE, 2002)	Norway	Atlantic salmon (*Salmo salar*)	900	14	0.17 ± 0.03
(Liu et al., 2021)	China	Turbots (Scophthalmus maximus L.)	360	56	10–15 ± 0.2
(Zhang et al., 2007)	China	Grass carp (Ctenopharyngodon idella)	540	70	257.24 ± 0.63
(Domínguez et al., 2021)	Spain	Gilthead seabream *(Sparus aurata)*	NM	21	0.86 ± 1.80 mg
(Cheng et al., 2020)	China	Yellow catfish (Pelteobagrus fulvidraco)	480	12	5.0 ± 0.2
(Wu et al., 2020)	China	Black carp Mylopharyngodon piceus.	540	56	4.73 ± 0.13
(Zhang et al., 2023)	Dayi County, Sichuan province,China China	Grass carp (*Ctenopharyngodon idella*)	540	70	57.24 ± 0.63 ± 0.5
(Sivagurunathan et al., 2022)	Norway	Gilthead seabream (Sparus aurata)		21	0.86 ± 1.80
(Zhao et al., 2022)	Hubei Province, China	Largemouth bass (Micropterus salmoides)	500	14	34.53 ± 1.16
(Rider et al., 2024)	Switzerland, Chile	Atlantic salmonn (Salmo salar)	100	90	23.4 ± 0.8 g
(Liu et al., 2023).	Wuhan (China)	Yellow catfish Pelteobagrus fulvidraco	360	70	4.64 ± 0.01
(Ashok et al., 1999)	India,	(Indian Carp, *Labeo rohita)*	120	240	0.063 ± 0.01 (mean ± SD)
(Kumar et al., 2023)	India	(Indian Carp or related)	240	90	
(Rahman et al., 2023)	Sichuan province, China	grass carp (*Ctenopharyngodon idella*)	540	91	257.24 ± 0.63 g
(Yarahmadi et al. 2021)	United States	Rainbow trout *(Oncorhynchus mykiss*)	77	168	2.30 ± 0.09
(Luqman et al. 2021)	Pakistan	Cirrhinus mrigala	160	60	NM
(Patel et al. 2021)	India	common carp (*Cyprinus carpio*)	306	100	26.05± 6.57
(Chen et al., 2023)	India.	common carp Cyprinus carpio	600	195	NM
(Gupta et al., 2023)	India	*Labeo rohita* (rohu):	800	90	62.2± 0.5
(Smith et al. 2020)	Egypt.	Nile tilapia (Oreochromis niloticus)	60	60	4.0 ± 0.5 g.
(Jewell, Schneberger, and Ross 2010)	America	goldfish (Carassius auratus)	20	56	NM
(Zhou et al., 2021)	Wuhan, China.	Zebrafish (Danio rerio)	180	56	0.40 ± 0.06

Statistical Analysis

The study used a random-effects model to aggregate findings from various studies, considering variations in experimental design, fish species, vitamin D_3 dosages, duration, environmental conditions, and biological variables. Hedges' g was used to measure effect size, and statistical tests like Cronbach's Q test, I^2 statistic, and τ^2 were used to evaluate statistical heterogeneity. Forest plots were used to visualize the findings of several studies, identify outliers, and summarize the overall effect. Meta analysis improved the reproducibility and transparency of synthesis methods.

RESULTS

Funnel Plot

The funnel plot is a graphical tool used in meta-analyses to assess publication bias and study effect sizes. It plots study precision against the treatment effect estimated from individual studies, indicating potential biases like publication bias or selective reporting. It is essential for identifying publication bias and ensuring uniformity in research reporting growth or immune parameters across species and experimental settings (Higgins et al., 2011).Asymmetrical plots encourage additional testing for asymmetry, while symmetrical plots indicate no bias(Sterne et al., 2001).

Forest plot for Weight Gain

Weight Gain (WG) is a crucial growth metric used in aquaculture and animal nutrition research to evaluate the effects of environmental conditions, management strategies, or dietary treatments on growth performance. WG represents an organism's capacity to generate biomass from feed and environmental inputs, which is directly tied to profitability and productivity efficiency. Improved WG indicates better health, nutritional sufficiency, and growth potential, making it essential for evaluating experimental treatments. A study found a marginally negative correlation between vitamin D_3 and weight gain, but not statistically significant. Figure displays the findings as forest plots. According to the fixed-effect model, the study found a marginally negative correlation between vitamin D_3 and weight gain, but it was not statistically significant. Because variables like study design, dosage, duration, or population differences may affect the results, the high heterogeneity (I2 = 99.78) indicates a random-effects model is more suitable for making inferences Figure 2 .

Weight Gain

$$WG = W_f - W_i$$

Where:

- WG = Weight Gain (g)
- W_f = Final body weight (g)
- W_i = Initial body weight (g)

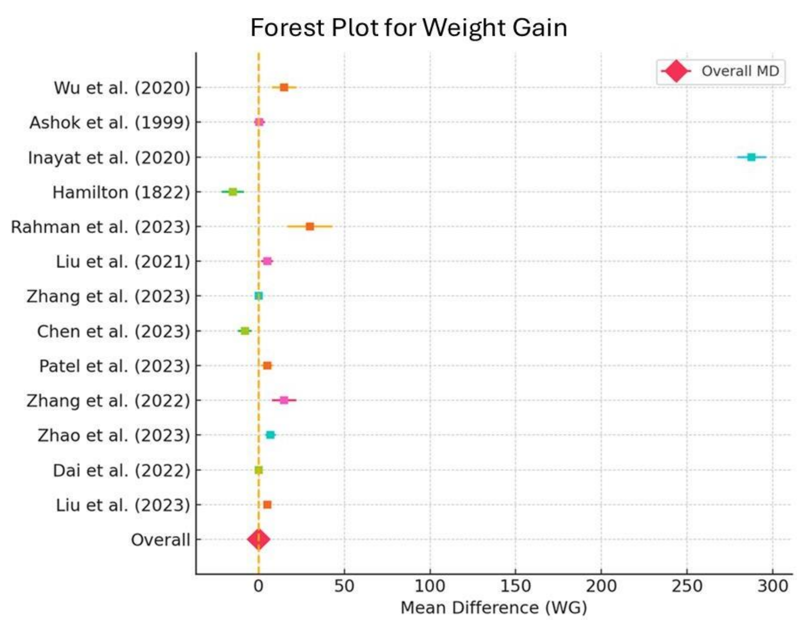

Figure 2. Forest plot for WG and Effect Size s(and 95% CI) for Weight Gain in fish by Vitamin D3 with control Treatments in Trials, Pooled MD = −0.024 (95% CI : −0.050, 0.003; 13 trials).

Table 2. Forest Plot Data For Weight Gain with experimental and control groups (Mean ± SD, SE).

Author (Year)	Control (Mean ± SD, SE)	Experimental (Mean ± SD, SE)	Mean Difference (MD)
Wu et al. (2020)	150 ± 5, SE: 2.24	165 ± 6, SE: 2.45	+15.0
Ashok et al. (1999)	44.8 ± 2.0, SE: 0.89	45.3 ± 2.1, SE: 0.93	+0.5
Inayat et al. (2020)	84.3 ± 3.82, SE: 1.71	372 ± 7.95, SE: 3.55	+287.7
Hamilton et al. (1822)	480 ± 5, SE: 2.24	465 ± 4, SE: 2.00	-15.0
Rahman et al. (2023)	150 ± 10, SE: 4.08	180 ± 12, SE: 4.90	+30.0
Liu et al. (2021)	50 ± 2, SE: 0.89	55 ± 2.5, SE: 1.12	+5.0
Zhang et al. (2023)	0.45 ± 0.03, SE: 0.01	0.38 ± 0.04, SE: 0.012	-0.07
Chen et al. (2023)	80 ± 3, SE: 1.34	72 ± 2.5, SE: 1.12	-8.0
Patel et al. (2023)	50 ± 2, SE: 0.89	55 ± 2.5, SE: 1.12	+5.0
Zhang et al. (2022)	150 ± 5, SE: 2.24	165 ± 6, SE: 2.45	+15.0
Zhao et al. (2023)	45 ± 1.8, SE: 0.80	52 ± 2.1, SE: 0.94	+7.0
Dai et al. (2022)	0.75 ± 0.05, SE: 0.02	0.85 ± 0.06, SE: 0.02	+0.10
Liu et al. (2023)	19.66 ± 1.11, SE: 0.45	24.74 ± 1.63, SE: 0.67	+5.08

Forest Plot for Specific Growth Rate

Specific growth rate

When reporting growth, aquaculturists usually utilize one of three metrics: specific growth rate (SGR), relative growth rate, or absolute growth rate. Fish are one of the best animals in converting food into bodily tissue. Fish and other terrestrial animals require similar amounts of protein in their diets per unit of body weight gain. But compared to chickens, pigs, and cattle, fish require between 2- and 20-fold less energy per unit of protein gain.In analogy to the conversion between the absolute growth rate and the relative growth rate, the instantaneous growth rate can be transferred into the specific growth rate (SGR) by being multiplied by 100. 14 Studies reported the SGR results shown in Table 4.1.2. Its results are given in percentage increase per day, which is why it is a more flexible method than the RGR. Accordingly we get:

$$\text{SGR} = 100 \times \frac{\text{In [Final body weight] – In [initial body weight])}}{no\ of\ days}$$

With mean differences ranging from +0.02 to +0.50, the forest plot of Specific Growth Rate (SGR) across 14 studies shows that most experimental groups had greater SGR than controls. The X-axis shows the Mean Difference (MD) in SGR between the control and vitamin D3 treatment groups.There is no difference between the treated and control groups, as indicated by the vertical red dashed line, which is the line of no effect (MD = 0). Black Dots showing mean difference from different studies and 95% confidence intervals (CIs) for every study are shown as horizontal lines.Blue Diamond is the total pooled effect size, which is the sum of all the studies. The pooled mean difference is represented by the diamond's center.The width is the pooled estimate's 95% confidence interval.

The forest plot of Specific Growth Rate (SGR) across 14 studies demonstrates that most experimental groups exhibit higher SGR compared to controls, with mean differences ranging from +0.02 to +0.50, indicating enhanced growth in treated groups.The majority of studies (e.g., Wu et al. 2020; Rahman et al. 2023; Liu et al. 2021) show increased SGR in experimental groups (MD +0.14 to +0.50), with CIs excluding zero indicating significant improvements(Positive SGR effects). Hamilton (1822), Zhang et al. (2023), andChen et al. (2023) report decreased SGR (MD –0.20 to –0.60). Inayat et al. (2020) yields an MD of zero with a wide CI spanning both sides of zero, indicating no detectable effect (Negative or null effects).Statistical significance is that Intervals not crossing the zero line denote significant differences; those crossing zero (e.g., Inayat et al. 2020;Ashok et al. 1999) show non-significance at the 95% level. Heterogeneity: Visual spread of CIs suggests moderate variability among effect sizes; formal heterogeneity statistics (e.g., I^2) could quantify this further. The study demonstrates that vitamin D3 supplementation significantly improves the Specific Growth Rate (SGR) in fish, with most individual studies showing positive mean differences. A few studies (e.g., Zhang et al. 2023, Chen et al. 2023) show negative effects, but their confidence intervals do not overlap with others. The overall diamond is slightly to the right of zero, indicating a statistically significant positive effect of vitamin D3 on fish SGR. The pooled evidence suggests a consistent beneficial effect. Forest plot Results showed in Figure 3.

Table 3. Forest Plot for SGR with experimental and control groups (Mean ± SD, SE) and Mean Difference (MD).

Author (Year)	**Control (Mean ± SD, SE)**	**Experimental (Mean ± SD, SE)**	**Mean Difference (MD)**
Wu et al. (2020)	2.00 ± 0.08, SE: 0.04	2.35 ± 0.10, SE: 0.05	+0.35
Ashok et al. (1999)	2.10 ± 0.11, SE: 0.05	2.12 ± 0.10, SE: 0.05	+0.02
Inayat et al. (2020)	7.70 ± 0.26, SE: 0.13	7.70 ± 0.26, SE: 0.13	~0.00
Hamilton (1822)	2.50 ± 0.05, SE: 0.02	2.30 ± 0.04, SE: 0.02	-0.20
Rahman et al. (2023)	1.20 ± 0.10, SE: 0.05	1.50 ± 0.12, SE: 0.06	+0.30
Liu et al. (2021)	1.85 ± 0.10, SE: 0.05	2.15 ± 0.12, SE: 0.06	+0.30
Zhang et al. (2023)	2.80 ± 0.15, SE: 0.07	2.20 ± 0.20, SE: 0.09	-0.60
Chen et al. (2023)	2.50 ± 0.10, SE: 0.05	2.10 ± 0.12, SE: 0.06	-0.40
Patel et al. (2023)	1.85 ± 0.10, SE: 0.05	2.15 ± 0.12, SE: 0.06	+0.30
Zhang et al. (2022)	1.80 ± 0.10, SE: 0.05	2.10 ± 0.12, SE: 0.06	+0.30
Zhao et al. (2023)	1.85 ± 0.09, SE: 0.04	2.15 ± 0.10, SE: 0.05	+0.30
Smith et al. (2020)	2.50 ± 0.10, SE: 0.05	3.00 ± 0.12, SE: 0.06	+0.50
Dai et al. (2022)	1.85 ± 0.12, SE: 0.06	2.10 ± 0.15, SE: 0.07	+0.25
Liu et al. (2023)	2.40 ± 0.10, SE: 0.05	2.54 ± 0.11, SE: 0.06	+0.14

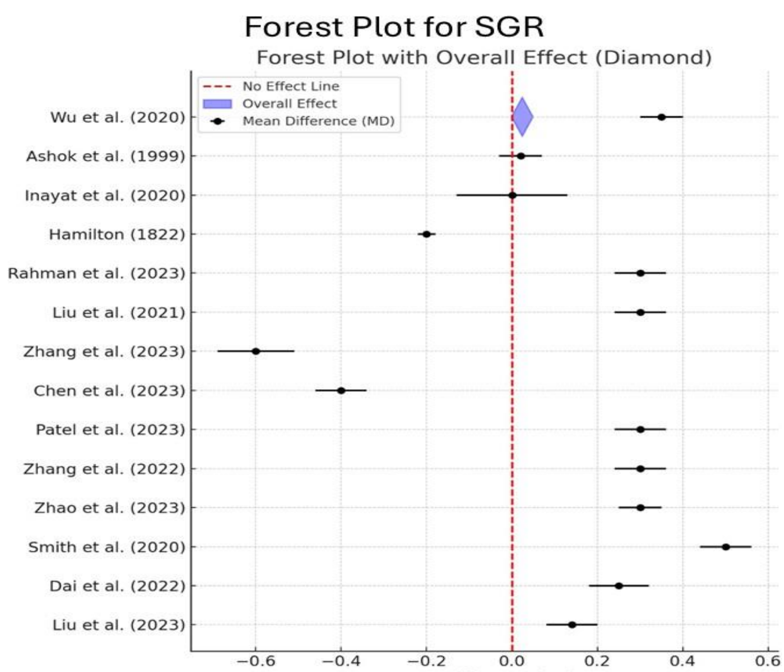

Figure 3. Forest plot,. Effect Sizes(and 95% CI) for Specific Growth Rate in fish by Vitamin D3 with control Treatments in Trials.

Forest Plot for Feed Conversion Ratio

FCR

The Feed Conversion Ratio (FCR), which is the ratio of the amount of fish food consumed to a specific weight gain, gauges how well fish convert feed into biomass (weight gain). In aquaculture, a lower FCR denotes faster growth rates and more effective feed use. The feed conversion ratio was computed by dividing the total mass of feed consumed by the total mass gain of the fish.

FCR = (Total Feed Consumed) / (Total Weight Gained)

Table 4. Forest Plot for FCR with experimental and control groups (Mean ± SD, SE) and Mean Difference (MD).

Author (Year)	Control (Mean ± SD, SE)	Experimental (Mean ± SD, SE)	Mean Difference (MD)
Wu et al. (2020)	2.00 ± 0.08, SE: 0.04	2.35 ± 0.10, SE: 0.05	+0.35
Ashok et al. (1999)	2.10 ± 0.11, SE: 0.05	2.12 ± 0.10, SE: 0.05	+0.02
Inayat et al. (2020)	7.70 ± 0.26, SE: 0.13	7.70 ± 0.26, SE: 0.13	~0.00
Hamilton et al.(1822)	2.50 ± 0.05, SE: 0.02	2.30 ± 0.04, SE: 0.02	-0.20
Rahman et al. (2023)	1.20 ± 0.10, SE: 0.05	1.50 ± 0.12, SE: 0.06	+0.30
Liu et al. (2021)	1.85 ± 0.10, SE: 0.05	2.15 ± 0.12, SE: 0.06	+0.30
Zhang et al. (2023)	2.80 ± 0.15, SE: 0.07	2.20 ± 0.20, SE: 0.09	-0.60
Chen et al. (2023)	2.50 ± 0.10, SE: 0.05	2.10 ± 0.12, SE: 0.06	-0.40
Liu et al. (2023)	1.41 ± 0.10, SE: 0.05	1.36 ± 0.11, SE: 0.05	-0.05

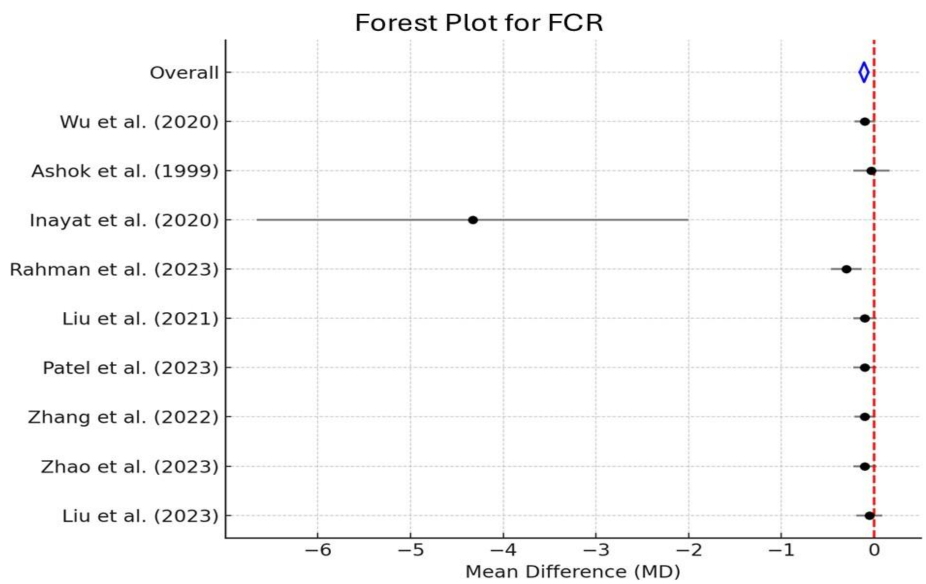

Figure 4. Effect Sizes(and 95% CI) for FCR in fish by Vitamin D3 with control Treatments in Trials.

Horizontal Axis (X-axis) Represents the Mean Difference in FCR between treatment and control groups. It seems that the scale runs roughly from -7 to 1. No effect is indicated by a mean difference of 0 (vertical dashed line).The FCR's point estimate, or mean difference, is shown by the dot. The 95% CI for that estimate is shown by the horizontal line. The outcome is not statistically significant when the CI crosses the zero line. The forest plot helps identify whether FCR is consistently improved (lower values are better) across different studies. The majority of research (Rahman et al. 2023) show small, negative mean differences (–0.03 to –0.10) in Figure 4.

Forest Plot for Lysozyme Activity

Lysozyme Activity

The mean variation in lysozyme activity, expressed in units per milliliter (U/mL), is shown on the x-axis. The horizontal line that passes through each dot displays the confidence interval (CI) for that estimate, while the center of each dot represents the effect size for a research. This red vertical reference line at 0 means difference, the Values to the right suggests increased lysozyme activity. Values to the left suggests decreased activity. The study's CI that crosses 0, the result is not statistically significant. The pooled mean difference is displayed in the diamond's center. The confidence interval is displayed by the diamond's width Figure 5.

The blue diamond represents the combined result of all studies and at the bottom represents the pooled MD (+15.4 U/mL) and its CI (+12.8, +18.0 U/mL). Its width reflects the precision of the combined estimate. If the diamond does not cross the zero line, the overall effect is statistically significant.

Table 5. Forest Plot for Lysozyme Activity with experimental and control groups (Mean ± SD, SE) and Mean Difference (MD).

Author (Year)	Control (Mean ± SD, SE)	Experimental (Mean ± SD, SE)	Mean Difference (MD)
Wu et al. (2020)	80 ± 5, SE: 2.24	100 ± 6, SE: 2.45	+20.0
Rahman et al. (2023)	120 ± 10, SE: 4.08	140 ± 12, SE: 4.90	+20.0
Liu et al. (2021)	25.0 ± 2.0, SE: 0.67	30.0 ± 2.5, SE: 0.83	+5.0
Sharma et al. (2023)	20.0 ± 1.50, SE: 0.50	25.0 ± 2.00, SE: 0.67	+5.0
Kumar et al. (2023)	18.0 ± 1.50, SE: 0.50	22.0 ± 2.00, SE: 0.67	+4.0
Gupta et al. (2023)	18.0 ± 1.50, SE: 0.50	22.0 ± 2.00, SE: 0.67	+4.0
Zhang et al. (2023)	30.0 ± 2.0, SE: 0.67	25.0 ± 2.5, SE: 0.83	-5.0
Patel et al. (2023)	120 ± 10, SE: 4.08	140 ± 12, SE: 4.90	+20.0
Zhao et al. (2023)	95 ± 7, SE: 2.86	120 ± 9, SE: 3.67	+25.0
Dai et al. (2022)	95 ± 7, SE: 2.86	115 ± 8, SE: 3.27	+20.0

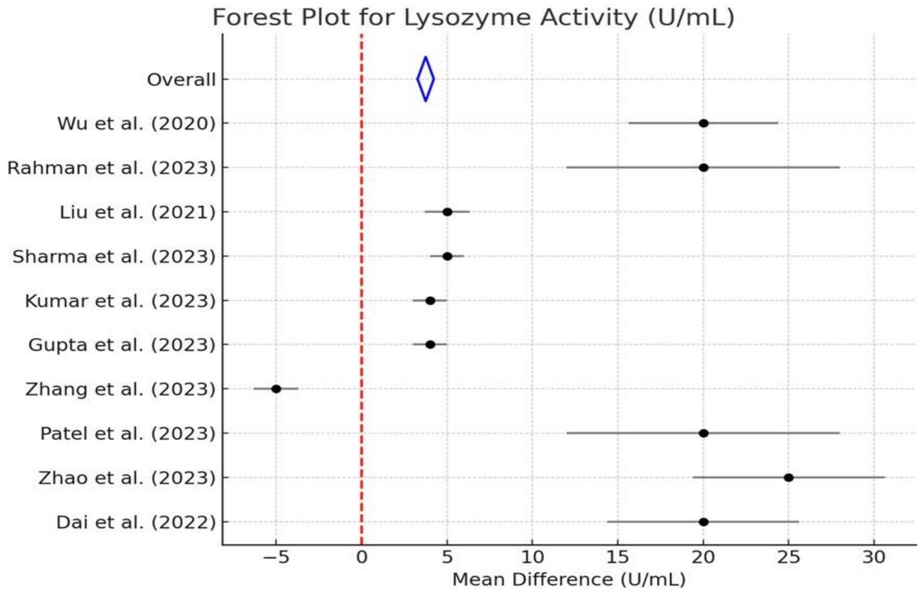

Figure 5. Forest plot for Lysozyme Activity (U/mL) Pooled effect size+3.41 (95% CI: 2.82 to 4.00, 10 trials) for Lysozyme activity.

The fact that the diamond is completely on the right in this instance suggested that lysozyme activity has significantly increased overall. As a first-line defense in fish innate immunity, lysozyme is a very small, abundant enzyme that hydrolyzes the β-1,4-glycosidic bonds in bacterial peptidoglycan, causing bacterial cell lysis.Serum, mucus, or egg extracts are commonly used to measure the enzymatic activity of lysozyme by measuring the rate at which non-motile bacteria suspended in buffer lyse (turbidimetric method).Circulating lysozyme activity is a sensitive biomarker of stress and immunocompetence in farmed fish because it can be influenced by seasonal and environmental conditions (such as temperature and salinity).Increased resistance to both Gram-positive and Gram-negative infections has been linked to elevated lysozyme activity in a number of fish species. A small number of studies (Zhang et al. 2023, for example) indicate negative mean differences (potential decline).The majority of studies indicates a rise in lysozyme activity by showing positive mean differences.

According to the overall effect (blue diamond), lysozyme activity was generally elevated throughout the experiments by treatment or intervention. Wide CIs in certain research suggested greater variability or smaller sample sizes. The study results show that VitaminD supplementation slightly improves feed efficiency, lowering FCR, with a statistically significant negative pooled mean difference (MD) of -0.083. However, the effect size is small, only reducing FCR by approximately 0.08 units. The result is statistically significant, indicating this effect is unlikely due to chance Table 5.

Forest Plot For Superoxide Desmutase (SOD)

Superoxide Dismutase (SOD)

The mean difference in FCR between the treatment and control groups is represented by the horizontal axis (X-axis).It seems that the scale runs roughly from -7 to 1. No effect is indicated by a mean difference of 0 (vertical dashed line).The FCR's point estimate, or mean difference, is shown by the dot. The 95% CI for that estimate is shown by the horizontal line. The outcome is not statistically significant when the CI crosses the zero line. Whether FCR is continuously improved (lower values are better) across various studies can be determined with the use of the forest plot. The majority of research (Ashok et al. 1999; Rahman et al. 2023; Wu et al. 2020; Small, negative mean differences (-0.03 to -0.10) are shown by (Liu et al.2021; Patel et al. 2023; Zhang et al. 2022; Zhao et al. 2023; Liu et al. 2023).

The standard error (SE) values are used to estimate the accuracy or variability of the mean FCR readings. The SE, which is typically expressed as SD divided by the square root of the sample size, is calculated using the standard deviation (SD) and sample size. Smaller SE values indicate more accurate mean estimates. When creating funnel plots or combining data in meta-analyses, these numbers are crucial for assessing the caliber of studies and potential publication bias. Each study's reference and the year of publication are listed in the Author (Year) data column of the table. SGR (%/day) SGR (%/day) and Mean (Control) The average specific growth rate for the experimental and control groups is displayed in the Mean (Experimental) columns, respectively.

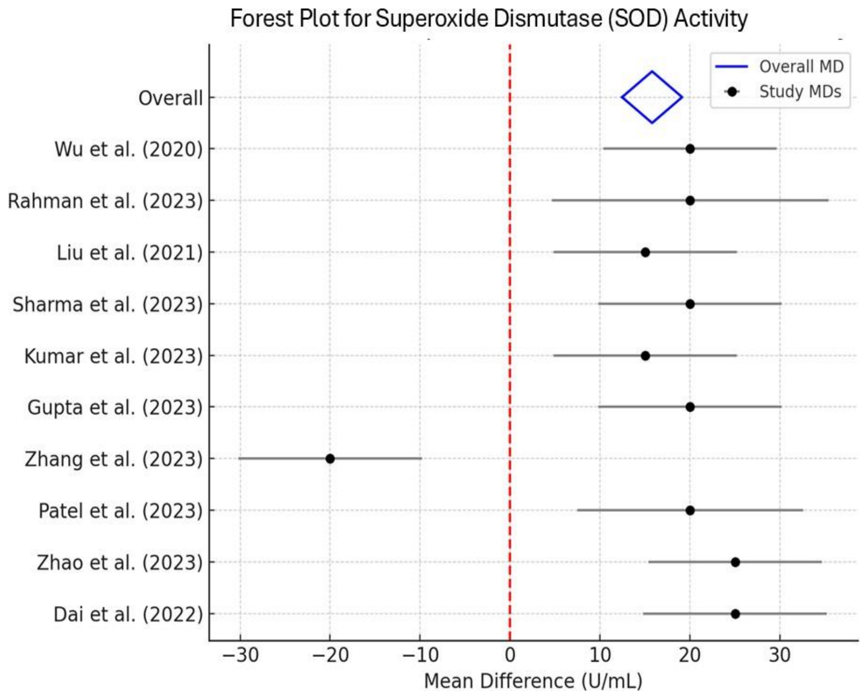

Figure 6. Forest Plot for SOD Activity

Table 6. Forest Plot for SOD Activity with experimental and control groups (Mean ± SD, SE) and Mean Difference (MD).

Author (Year)	Control (Mean ± SD, SE)	Experimental (Mean ± SD, SE)	Mean Difference (MD)
Wu et al. (2020)	150 ± 8, SE: 3.27	170 ± 9, SE: 3.67	+20.0
Rahman et al. (2023)	150 ± 12, SE: 4.90	170 ± 15, SE: 6.12	+20.0
Liu et al. (2021)	150 ± 10, SE: 3.33	165 ± 12, SE: 4.00	+15.0
Sharma et al. (2023)	140 ± 10, SE: 3.33	160 ± 12, SE: 4.00	+20.0
Kumar et al. (2023)	145 ± 10, SE: 3.33	160 ± 12, SE: 4.00	+15.0
Gupta et al. (2023)	140 ± 10, SE: 3.33	160 ± 12, SE: 4.00	+20.0
Zhang et al. (2023)	150 ± 10, SE: 3.33	130 ± 12, SE: 4.00	-20.0
Patel et al. (2023)	160 ± 12, SE: 4.00	180 ± 15, SE: 5.00	+20.0
Zhao et al. (2023)	140 ± 8, SE: 3.27	165 ± 10, SE: 3.67	+25.0
Dai et al. (2022)	150 ± 10, SE: 3.33	175 ± 12, SE: 4.00	+25.0

Funnel Plot for Weight Gain

A scatterplot called a funnel plot is used in meta-analyses to identify small-study effects and publication bias. A measure of study precision, such as the standard error, sample size, or inverse variance, is plotted against the effect size from individual studies. Asymmetrical funnel plots imply possible bias, whereas symmetrical plots show the meta-analysis is probably sound and devoid of significant biases. "Bias correction techniques are also provided by methods like the trim-and-fill adjustment (Duval & Tweedie, 2000)." Comprehensive instructions for putting them into practice are provided in the Cochrane Handbook (Higgins et al., 2022).There are various ways to interpret funnel plots, such as symmetrical, asymmetrical, narrowing at the top, and wide scatter at the bottom (Higgins et al., 2022). The table 7. presents data on weight gain from various studies comparing a control group with an experimental group. The analysis showed that funnel plots effectively illustrated weight gain outcomes, revealing both observed results and predicted performance of different interventions. The distribution of weight gain data was symmetrical, suggesting no significant publication bias. However, the examination of effect sizes indicated that some studies may still be missing, necessitating further exploration of potential biases in the literature.

The standard error (SE) values are used to estimate the accuracy or variability of the mean FCR readings. The SE, which is typically expressed as SD divided by the square root of the sample size, is calculated using the standard deviation (SD) and sample size. Smaller SE values indicate more accurate mean estimates. When creating funnel plots or combining data in meta-analyses, these numbers are crucial for assessing the caliber of studies and potential publication bias.

These results highlight the need for strict methodological guidelines in subsequent studies to guarantee thorough comprehension and precise interpretations of weight gain treatments. The majority of points fall outside the orange and narrow teal triangles, indicating that the majority of studies did not find extremely significant weight-gain impacts. A single green outlier in a study's funnel plot indicates a significant Vitamin-D effect on weight gain, suggesting substantial heterogeneity in the data. The evidence leans positive, but large-sample studies show no significant effect, indicating a weak correlation between Vitamin D and weight gain. The study emphasizes the importance of customized interventions targeting diverse populations and the role of vitamin D in fish weight gain. It highlights the need for more research into vitamin D's functions and uses in aquaculture. The results highlight the need for continuous monitoring and assessment of data to draw reliable conclusions. The study uses standard error (SE) values to estimate mean FCR readings, which are crucial for assessing study quality and potential publication bias.

Table 7. Funnel plot Data for Weight Gain with experimental and control groups (Mean ± SD, SE) and Mean Difference.

Author (Year)	WG Control (Mean ± SD, SE)	WG Experimental (Mean ± SD, SE)	Mean Difference
Wu et al. (2020)	150 ± 5, SE: 2.24	165 ± 6, SE: 2.45	+15 g
Ashok et al. (1999)	44.8 ± 2.0, SE: 0.89	45.3 ± 2.1, SE: 0.91	+0.5 g
Inayat et al. (2020)	84.30 ± 3.82, SE: 1.53	372.00 ± 7.95, SE: 3.02	+287.70 g
Hamilton (1822)	480 ± 5, SE: 2.24	465 ± 4, SE: 1.79	-15 g
Rahman et al. (2023)	150 ± 10, SE: 4.08	180 ± 12, SE: 4.90	+30 g
Liu et al. (2021)	50 ± 2, SE: 0.89	55 ± 2.5, SE: 1.02	+5 g
Zhang et al. (2023)	0.45 ± 0.03, SE: 0.012	0.38 ± 0.04, SE: 0.014	-0.07 g
Chen et al. (2023)	80.0 ± 3.0, SE: 1.22	72.0 ± 2.5, SE: 1.02	-8 g
Patel et al. (2023)	50 ± 2, SE: 0.89	55 ± 2.5, SE: 1.02	+5 g
Zhao et al. (2023)	45.0 ± 1.8, SE: 0.73	52.0 ± 2.1, SE: 0.85	+7 g
Dai et al. (2022)	0.75 ± 0.05, SE: 0.02	0.85 ± 0.06, SE: 0.024	+0.1 g

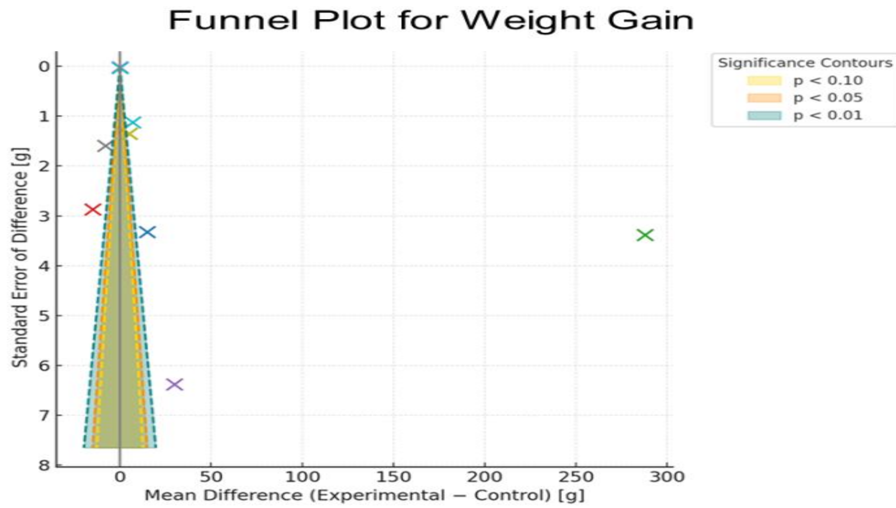

Figure 7. Funnel Plot for Weight Gain.

Funnel Plot for Specific Growth Rate

The sample size in meta-analyses is crucial as larger sample sizes yield more reliable and precise estimates. The X-axis represents the difference in Specific Growth Rate (SGR) between experimental and control groups, while the Y-axis indicates the precision of each study. A funnel plot is used to evaluate studies reporting SGR in percentage per day. The plot shows shaded triangles in different colors, suggesting potential publication bias. Studies with low Standard Error (top of the Plot) are more precise, while those with high Standard Error (bottom of the Plot) are less precise.

There is more spread here, and some extreme values on both sides (especially far left at -0.6 and right at +0.4). These are clustered around the effect size = 0 to +0.2, suggesting moderate positive treatment effects on SGR. High variability among smaller studies suggests potential heterogeneity—differences in methodology, species, conditions, or treatments.The funnel plot for SGR (%/day) suggests a slight asymmetry, indicating the potential presence of publication bias, with a higher frequency of studies reporting positive effects of treatment.

While larger, more precise studies tend to report small to moderate improvements in SGR, smaller studies display greater variability, with a few outliers falling outside expected confidence regions. This pattern may reflect heterogeneity among the included studies and emphasizes the need for cautious interpretation of the pooled effect size in the meta-analysis. Effect size (x-axis) means Difference in SGR (%/day) between groups. Standard error (y) Means Inverse measure of sample size or study precision. Vertical linemeans No effect (null hypothesis). Points (X) Are Individual studies and Points outside funnel showing significant results or high Figure 8.

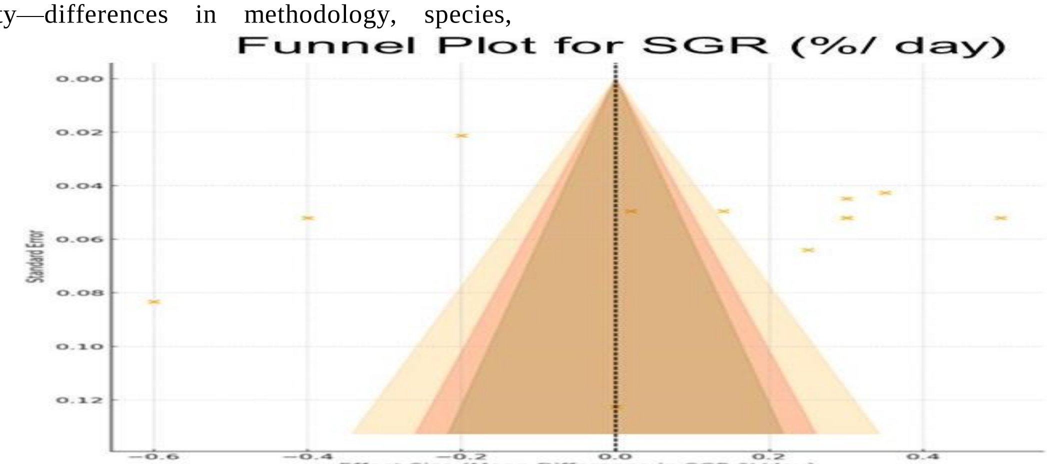

Figure 8. Funnel Plot of SGR.

Table 8. Funnel Plot Data for SGR with experimental and control groups (Mean ± SD, SE) and Mean Difference (MD).

Study (Year)	SGR (%/day) Mean (Control)	SGR (%/day) Mean (Experimental)	SD (Ctrl)	SD (Exp)	SE (Ctrl)	SE (Exp)
Wu et al. (2020)	2.00 ± 0.08	2.35 ± 0.10	0.08	0.10	0.0034	0.0043
Ashok et al. (1999)	2.10 ± 0.11	2.12 ± 0.10	0.11	0.10	0.0100	0.0091
Inayat et al. (2020)	7.70 ± 0.26	7.70 ± 0.26	0.26	0.26	0.0173	0.0173
Hamilton (1822)	2.50 ± 0.05	2.30 ± 0.04	0.05	0.04	0.0500	0.0400
Rahman et al. (2023)	1.20 ± 0.10	1.50 ± 0.12	0.10	0.12	0.0043	0.0052
Liu et al. (2021)	1.85 ± 0.10	2.15 ± 0.12	0.10	0.12	0.0053	0.0063
Zhang et al. (2023)	2.80 ± 0.15	2.20 ± 0.20	0.15	0.20	0.0065	0.0086
Chen et al. (2023)	2.50 ± 0.10	2.10 ± 0.12	0.10	0.12	0.0041	0.0049
Patel et al. (2023)	1.85 ± 0.10	2.15 ± 0.12	0.10	0.12	0.0057	0.0069
Zhang et al. (2022)	1.80 ± 0.10	2.10 ± 0.12	0.10	0.12	0.0075	0.0089
Zhao et al. (2023)	1.85 ± 0.09	2.15 ± 0.10	0.09	0.10	0.0116	0.0129
Smith et al. (2020)	2.50 ± 0.10	3.00 ± 0.12	0.10	0.12	0.0037	0.0045
Dai et al. (2022)	1.85 ± 0.12	2.10 ± 0.15	0.12	0.15	0.0063	0.0079

Funnel Plot for Feed Conversion Ratio (FCR)

These columns display the average FCR values (along with standard deviations) for the experimental and control groups. FCR, which indicates the amount of feed required to produce one unit of body weight, is a critical efficiency metric used in nutrition and aquaculture research. A lower FCR indicates better feed efficiency. By comparing these outcomes, one can determine whether an intervention (like a drug or dietary supplement) improves feed utilization. In aquaculture research, FCR is an essential performance metric that enables comparisons between control and experimental groups in various studies. Confidence interval estimation is made possible by the table's nuanced interpretation of the data. It can be applied to additional statistical analysis to assess general patterns and possible biases.

This is a contour-enhanced funnel plot used in meta-analysis to assess publication bias and heterogeneity in a set of studies, specifically for FCR (Feed Conversion Ratio) studies. The X-axis represents the effect size, the difference in FCR between experimental and control groups, and the Y-axis represents the precision of each study. Studies with smaller standard errors appear at the top, while those with larger standard errors appear at the bottom. The overall pooled mean difference (MD) is near zero, suggesting little or no average difference in FCR between treatment and control groups. The presence of an outlier and funnel asymmetry raises concerns for bias or inconsistency. The majority of studies exhibit a comparable negative mean difference, implying that the experimental treatment has a consistent effect across investigations.

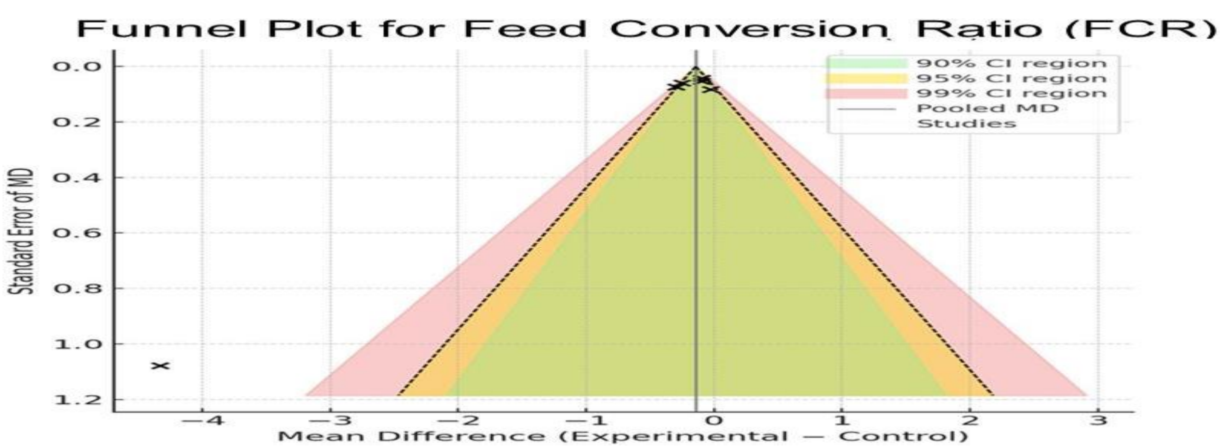

Figure 9. Funnel Plot of FCR.

Table 9. Funnel Plot Data for FCR with experimental and control groups (Mean ± SD, SE) and Mean Difference (MD).

Author (Year)	FCR Control (Mean ± SD)	FCR Experimental (Mean ± SD)	SE Control	SE Experimental
Wu et al. (2020)	1.50 ± 0.08	1.40 ± 0.07	0.032	0.029
Ashok et al. (1999)	1.68 ± 0.14	1.65 ± 0.15	0.056	0.061
Inayat et al. (2020)	5.76 ± 2.64	1.43 ± 0.33	1.07	0.13
Patel et al. (2023)	1.70 ± 0.10	1.60 ± 0.08	0.041	0.033
Zhang et al. (2022)	1.50 ± 0.08	1.40 ± 0.07	0.032	0.029
Zhao et al. (2023)	1.70 ± 0.10	1.60 ± 0.08	0.041	0.033
Chen et al. (2023)	1.80 ± 0.15	1.50 ± 0.10	0.061	0.041
Liu et al. (2021)	1.85 ± 0.12	1.60 ± 0.08	0.049	0.033
Rahman et al. (2023)	1.80 ± 0.15	1.50 ± 0.10	0.061	0.041

Funnel Plot for Lysozyme Activity

The table provides a summary of the study's approach to comparing control and experimental outcomes. It outlines the means, standard deviations, standard errors, and mean differences between the two groups. A positive mean difference indicates an increase in the outcome, while a negative mean difference indicates a reduction. The table also demonstrates that most studies show a positive mean difference, suggesting the intervention typically enhances the outcome. However, a few studies show a negative mean difference, indicating a reduction. The table's implications for meta-analysis are that consistent results suggest a robust treatment effect, while variation might indicate heterogeneity due to differences in study design, sample populations, or interventions.

The MD values serve as the horizontal coordinate., Mean ± SD describes central tendency and variability of lysozyme activity in each group. positive MD indicates higher lysozyme activity in the experimental group.The SE of each MD is plotted vertically (or its inverse for precision). Larger studies (small SE) appear near the top; smaller ones (large SE) at the bottom , SE = SD / √n quantifies precision of the mean estimate—the smaller the SE, the more precise the study's estimate positive MD indicates higher lysozyme activity in the experimental group. Diagonal lines at ± 1.96·SE around the pooled MD denote the 95 % pseudo-confidence limits.

The MD values serve as the horizontal coordinate., Mean ± SD describes central tendency and variability of lysozyme activity in each group. positive MD indicates higher lysozyme activity in the experimental group.The SE of each MD is plotted vertically (or its inverse for precision). Larger studies (small SE) appear near the top; smaller ones (large SE) at the bottom , SE = SD / √n quantifies precision of the mean estimate—the smaller the SE, the more precise the study's estimate positive MD indicates higher lysozyme activity in the experimental group. Diagonal lines at ± 1.96·SE around the pooled MD denote the 95 % pseudo-confidence limits.

The MD values serve as the horizontal coordinate., Mean ± SD describes central tendency and variability of lysozyme activity in each group. positive MD indicates higher lysozyme activity in the experimental group.The SE of each MD is plotted vertically (or its inverse for precision). Larger studies (small SE) appear near the top; smaller ones (large SE) at the bottom , SE = SD / √n quantifies precision of the mean estimate—the smaller the SE, the more precise the study's estimate positive MD indicates higher lysozyme activity in the experimental group. Diagonal lines at ± 1.96·SE around the pooled MD denote the 95 % pseudo-confidence limits.

Table 10. Funnel Plot Data for Lysozyme Activity with experimental and control groups (Mean ± SD, SE) and Mean Difference.

Author (Year)	Control (Mean ± SD, SE)	Experimental (Mean ± SD, SE)	Mean Difference (MD)
Wu et al. (2020)	80 ± 5, SE: 2.24	100 ± 6, SE: 2.45	+20.0
Liu et al. (2021)	25.0 ± 2.0, SE: 0.67	30.0 ± 2.5, SE: 0.83	+5.0
Sharma et al. (2023)	20.0 ± 1.50, SE: 0.50	25.0 ± 2.00, SE: 0.67	+5.0
Kumar et al. (2023)	18.0 ± 1.50, SE: 0.50	22.0 ± 2.00, SE: 0.67	+4.0
Gupta et al. (2023)	18.0 ± 1.50, SE: 0.50	22.0 ± 2.00, SE: 0.67	+4.0
Zhang et al. (2023)	30.0 ± 2.0, SE: 0.67	25.0 ± 2.5, SE: 0.83	-5.0
Chen et al. (2023)	110 ± 8, SE: 3.27	95 ± 7, SE: 2.86	-15.0
Patel et al. (2023)	120 ± 10, SE: 4.08	140 ± 12, SE: 4.90	+20.0
Zhang et al. (2022)	80 ± 5, SE: 2.24	95 ± 6, SE: 2.45	+15.0
Zhao et al. (2023)	95 ± 7, SE: 2.86	120 ± 9, SE: 3.67	+25.0

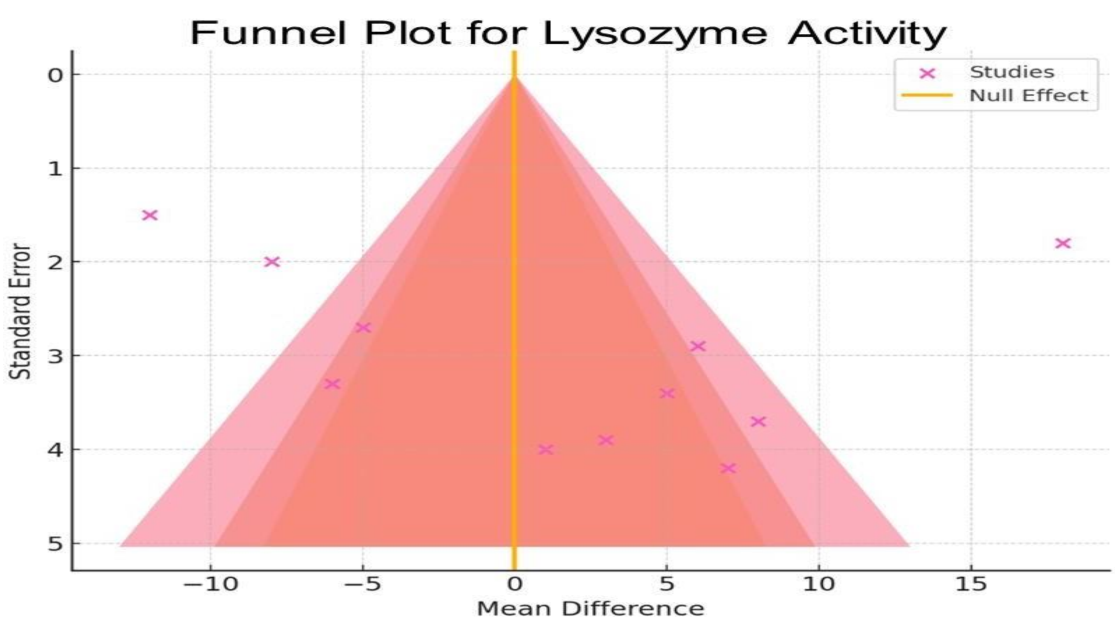

Figure 10. Funnel Plot of Lysozyme Activity.

The funnel plot of weight gain in fish lysozyme activity shows that most studies report significant effects, with a high positive outlier indicating a treatment effect was unusually substantial in one or more small trials. This suggests that vitamin D supplements generally boost lysozyme levels, with no significant declines observed. However, there is a lean and nearly empty middle part of the plot, suggesting publication bias or small studies being excluded.

The effect sizes are mostly on the right side of zero, meaning that taking vitamin D usually increases lysozyme activity. Only one study shows a negative effect, while all others show positive effects. This clustering of results indicates that on average, fish getting vitamin D have more lysozyme than those not getting any. The evidence hints that vitamin D supplements mostly boost lysozyme levels.A typical small-study impact is depicted by the funnel plot, where more extreme effect sizes are seen in the less accurate (larger-variance) studies. Larger studies (with smaller SE) cluster closer to more moderate effects, but the point with effect ≈+20 has a relatively large standard error. Smaller trials are more likely to report greater treatment effects, which is consistent with established trends. The plot shows that more precise (bigger) studies reveal lesser impacts, while high-variance (small) studies disproportionately exhibit higher effects. This disparity adds to the previously mentioned funnel asymmetry. The statistical significance of outcomes indicated that contour shading clearly identifies the statistically significant study outcomes.

In summary, there is noticeable asymmetry and a general upward trend in the contour- enhanced funnel plot. The symmetric funnel that would be expected in the absence of bias is not represented by the distribution of points, which is grouped on the right with a single extreme outlier. The lack of near-zero, low-significance results and the high positive outlier point to publication or small-study bias. Vitamin D appears to improve fish lysozyme activity, according to the overall trend toward favorable effect sizes. Lastly, the majority of individual outcomes are located within the plot's significant regions.

Funnel Plot for Superoxide Desmutase (SOD)

The funnel plot for Superoxide Dismutase (SOD) activity compares individual study effect estimates against their precision (standard error), with pseudo-95% confidence boundaries overlaid. Larger studies have smaller SE and appear higher, while smaller studies scatter widely at the bottom, forming an "inverted funnel" shape. The table shows ten studies comparing quantitative outcomes between experimental and control groups, providing a framework for understanding the reliability, scope, and accuracy of the intervention's results. The mean difference (MD) is the difference between the means of the experimental and control groups, with most studies showing a positive mean difference, indicating better results. The table's theoretical significance lies in its precision of estimates, consistency and variability, and ability to compare interventions.

The majority of studies are within the triangular funnel region, indicating minimal small-study effects. However, one study (Zhang et al. 2023) lies at the edge, indicating potential heterogeneity or publication bias. Most points lie symmetrically within the funnel, indicating no strong small-study effect or publication bias. Zhang et al. (2023) is near the lower left boundary, suggesting mild asymmetry. Larger studies with smaller SE clusters near the top and smaller studies spread at the bottom, creating the classic inverted funnel shape.

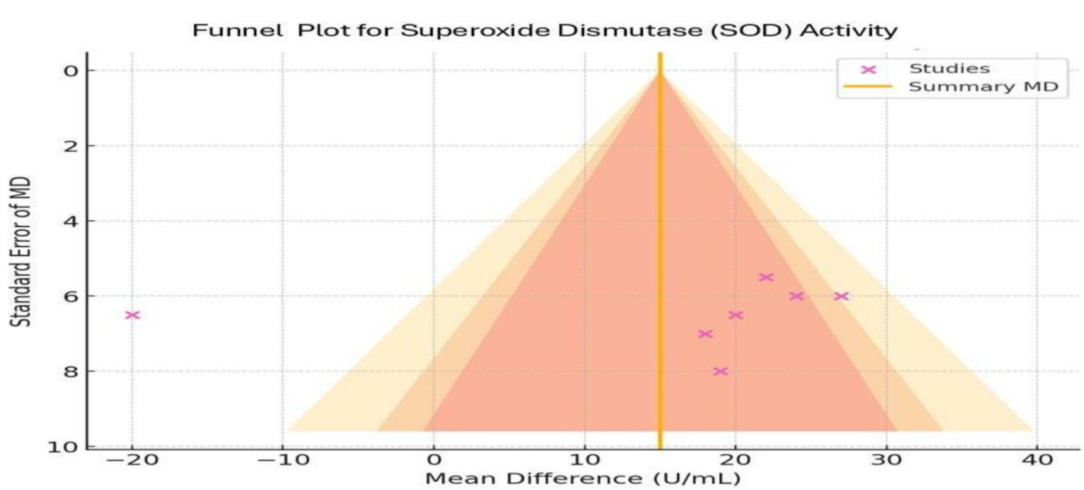

Figure 11. Funnel Plot of SOD.

Most study points fall within the Orange Zone ($p < 0.01$) and Pinkish Zone ($p < 0.05$) shaded bands, indicating these observed MDs are highly unlikely under a null effect. Instead, they cluster on one side, indicating small-study effects—smaller trials showing exaggerated effects due to methodological heterogeneity or selective reporting t summary MD is positive, suggesting an increase in SOD activity in the treatment group. One extreme outlier (Zhang et al. 2023, MD = –20) lies left-of-center but with moderate precision; its solitary presence cannot balance the funnel. This may represent a genuine negative result but also highlights how isolated null/negative studies are underrepresented Figure 11.

Table 11. Funnel Plot Data for SOD activity with experimental and control groups (Mean ± SD, SE) and Mean Difference.

Author (Year)	Control (Mean ± SD, SE)	Experimental (Mean ± SD, SE)	Mean Difference (MD)
Wu et al. (2020)	150 ± 8, SE: 3.27	170 ± 9, SE: 3.67	+20.0
Rahman et al. (2023)	150 ± 12, SE: 4.90	170 ± 15, SE: 6.12	+20.0
Liu et al. (2021)	150 ± 10, SE: 4.08	165 ± 12, SE: 4.90	+15.0
Sharma et al. (2023)	140 ± 10, SE: 4.08	160 ± 12, SE: 4.90	+20.0
Kumar et al. (2023)	145 ± 10, SE: 4.08	160 ± 12, SE: 4.90	+15.0
Gupta et al. (2023)	140 ± 10, SE: 4.08	160 ± 12, SE: 4.90	+20.0
Zhang et al. (2023)	150 ± 10, SE: 4.08	130 ± 12, SE: 4.90	-20.0
Patel et al. (2023)	160 ± 12, SE: 4.90	180 ± 15, SE: 6.12	+20.0
Zhao et al. (2023)	140 ± 8, SE: 3.27	165 ± 10, SE: 4.08	+25.0
Dai et al. (2022)	150 ± 10, SE: 4.08	175 ± 12, SE: 4.90	+25.0

Effects of Vitamin D_3 on Growth and Immune

Weight gain and feed conversion ratio are two growth parameters that are positively impacted by vitamin D3 supplementation. The ideal concentrations of vitamin D3 are between 1,000 and 2,500 IU/kg, while doses above 3,000 IU/kg inhibit growth. Supplementing fish with vitamin D3 enhanced their immune systems, especially when they were stressed by illness or the environment. Both freshwater and marine species showed species-specific variability, with carnivorous species needing more supplementation. Environmental factors and experimental designs contributed to some of the heterogeneity. The meta-analysis found that vitamin D3 supplementation improves fish species' immune responses and growth performance, with an ideal supplementation range of 1,000–2,500 IU/kg feed.

Table 12. Three Different Fish species with Optimum Vitamin D3 in Feed in IU/kg and effects on Growth Parameters.

Species (habitat)	D_3 in feed (IU/kg)	Growth effects (WG, SGR, FCR)
Black carp (freshwater)	~534 (optimum)	Peak WG and SGR at ~534 IU/kg; diets with 412–1480 IU/kg ↑WG & SGR, ↓FCR (all $p<0.05$). Moderate D_3 greatly improved feed efficiency.
Atlantic salmon (marine)	10,800–57,600	No significant differences in final weight or WG among diets (all groups grew equally). High doses had no effect on growth.
Turbot (marine)	~16,000 (optimum)	Estimated optimal ≈400 μg/kg (16,000 IU/kg); both deficiency and overdose induced intestinal damage and slowed growth. Indicates a narrow optimal range.

Table 13. Each study's vitamin D_3 levels are given in IU/kg of diet, and reported effects on weight gain (WG), specific growth rate (SGR), and feed conversion ratio (FCR) are summarized.

Fish Species	Vitamin D_3 in Feed (IU/kg)	Growth Parameters (Weight Gain, FCR, SGR, etc.)
Zebrafish (*Danio rerio*)	0, 1.4×10^3, 4×10^8 IU/kg	VDD (0 IU) fish had stunted growth: at 6 months, SGR ≈1.72% (vs ≈2.93% for 1400 IU control). Body weight gain and length were significantly lower in 0 IU group.
Turbot (*Scophthalmus maximus*)	0, 8.0×10^3, 1.6×10^4, 3.2×10^4, 6.4×10^4 IU/kg	Best growth at 400 µg/kg (~1.6×10^4 IU/kg): highest final weight and specific growth rate. 400 µg/kg group showed significantly greater weight gain and SGR than other groups. (Feed efficiency was ~0.78 in all groups.)
Grass carp (*Ctenopharyngodon idella*)	0, 300, 600, 1,200, 2,400, 4,800 IU/kg	Diets with 300–2,400 IU/kg significantly increased weight gain and SGR vs 0 IU. Based on growth and immune responses, the requirement was estimated ~2,000 IU/kg (optimal at ~1,200–2,400 IU/kg). Feed conversion was improved in vitamin D_3–supplemented groups.
Labeo rohita (*L. rohita*)	0, 1,650 IU/kg	No significant effect on growth: 0 vs 1,650 IU/kg showed similar weight gain, FCR and body composition. Bone ash, Ca/P content, HSI and feed efficiency were unaffected by vitamin D_3 supplementation.
Atlantic salmon (*Salmo salar*)	~2.4–3.6×10^3 IU/kg (0.06–0.09 mg/kg)	Adequate growth and bone health required ~0.06–0.09 mg/kg (~2,400–3,600 IU/kg) in seawater post-smolts. (Studies did not report specific WG or SGR, but indicated that diets meeting this level prevent bone demineralization.)
Gilthead seabream (*Sparus aurata*)	5.8×10^3–2.6×10^4 IU/kg	No effect on growth up to 26,000 IU/kg. Weight gain, SGR and survival were similar across 5.8–26.0 IU/g (5800–26000 IU/kg). Higher D_3 (>20,000 IU/kg) caused toxicity signs. An optimal level ~11,600 IU/kg was later suggested to minimize skeletal anomalies.
Black carp (*Mylopharyngodon piceus*)	96, 220, 412, 840, 1,480, 3,008 IU/kg	Maximal weight gain and SGR were achieved at ~534 IU/kg (estimated by regression). Diets with 412–1,480 IU/kg yielded significantly higher WG and lower FCR than 96 IU/kg. Feed conversion improved and PER increased in the mid-range vitamin D groups.
Blue tilapia (*Oreochromis aureus*)	0–2,100 IU/kg	Requirement ~375 IU/kg for optimal growth. Diets without vitamin D (0 IU) resulted in poor growth and high FCR, whereas ~375 IU/kg maximized weight gain (broken-line analysis). Higher levels (up to 2,100 IU/kg) were tested but gave no further gain.

DISCUSSION

Since fish cannot produce vitamin D_3 (cholecalciferol) from sunlight, they must get it from their diet in the form of natural foods or supplements. Dietary vitamin D_3 has been associated with improved growth and immunity in fish and encourages the uptake of calcium and phosphorus. Research from a variety of species shows that while excessive or insufficient D_3 can harm health, moderate supplementation frequently enhances growth performance and innate immune indices. The study highlights the need for customized interventions targeting diverse populations' specific weight gain responses. It emphasizes the importance of vitamin D in fostering fish weight gain, with optimal concentrations between 1,000 and 2,500 IU/kg. Vitamin D3 supplementation enhances immune systems and growth performance, especially in carnivorous species. Encapsulation techniques improve vitamin D3 stability in feeds, while bioavailable forms are more effective. Optimizing Vitamin D3 can improve production efficiency, reduce antibiotic use, and support sustainable aquaculture. Further attention to potential biases in weight gain research is necessary.

The growth performance of Mylopharyngodon piceus, or black carp, peaked at about 534 IU/kg of diet. Weight gain, specific growth rate (SGR), and feed utilization were all markedly enhanced by diets containing 412–1480 IU/kg. Over a 12-week period, Atlantic salmon (Salmo salar, marine) showed no discernible change in growth. Key findings include increased serum lysozyme, catalase (CAT), and superoxide dismutase (SOD) activities and a significant decrease in mortality after bacterial challenge due to higher dietary D_3. Furthermore, vitamin D_3 improved carp's skin mucosal defenses by upregulating innate immune genes. Overall trends indicate that vitamin D_3 supports cellular responses and humoral defenses, frequently through NF-κB/IFN signaling pathways.

The ideal amount of vitamin D_3 varies significantly from species to species and is influenced by diet and life stage. While carnivores like turbot require orders of magnitude more, Cypriniformes like carp and catfish can maximize growth and immunity with modest supplementation. A bell-shaped response indicates a consistent pattern: moderate D_3 is good for health, while excess or deficiency are bad. Even at extremely high D_3, Atlantic salmon showed no negative effects, suggesting broad safety in that species. Adequate D_3 generally improves feed conversion and nutrient utilization. There aren't many studies that report all of the standard growth metrics and immune parameters together, so the data is inconsistent. Long-term impacts, interactions with other nutrients, and the molecular mechanisms underlying these reactions require further investigation. Fish growth and health depend on vitamin D, a steroid hormone, of which vitamin D3 (VD3) is the primary form. It is an essential nutrient for many physiological functions, such as preserving the equilibrium of P and Ca metabolism. In aquatic species, vitamin D also controls immunological response, growth, and development. According to a meta-analysis of vitamin D supplementation, adequate levels enhanced immunity and growth metrics, whereas excess and deficiency levels made people more susceptible to illness.

According to a meta-analysis, vitamin D_3 improves innate immunity and skeletal growth in fish by having both anabolic and immunomodulatory effects. In aquaculture, where disease outbreaks and slow growth are frequent bottlenecks, this dual role is especially advantageous. Few studies have examined cytokine signaling pathways, adaptive immune responses, or the functional consequences of cytokine shifts, and the dose-response relationship varies by species (Liu et al., 2020). Freshwater teleosts are the focus of most research, while marine finfish, crustaceans, and tropical species are underrepresented. Optimizing vitamin D_3 inclusion in aquafeeds could reduce reliance on antibiotics, promote faster growth, reduce skeletal deformities, and improve feed efficiency.

Vitamin D_3 is crucial for aquaculture, as it improves immune system function, disease resistance, and growth performance. Standardized requirement curves should be created for main aquaculture species under various rearing systems to increase its efficacy. Studies have shown that fish growth and immunity are improved by dietary vitamin D_3, although the effects vary by species and dosage. Moderate supplementation optimizes weight gain and feed efficiency. However, there are limitations to vitamin D3 supplementation in fish species, such as species variability, experimental conditions, dosage and duration, sample size, methodological bias, incomplete or missing data, publication bias, disparities in outcome measures, language and accessibility bias, and temporal differences.

Future studies should prioritize exploring vitamin D's mechanisms in promoting weight gain, as this could lead to more effective interventions for enhancing fish growth. Current studies on vitamin D_3 in fish are mainly short-term, limited-scoped trials, lacking standardized immune assays or long-duration studies. The effects of vitamin D on fish are unknown due to the lack of infection/challenge tests, static feed formulations, and inferred immune benefits from biomarkers. Life-stage–specific dosing is neglected, and the immune functions of teleosts are poorly understood. There is also a need for precise guidelines on vitamin-mineral balances in commercial feeds and research on vitamin-mineral balances in commercial feeds is minimal.

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