



# Utilisation of Humic Substances as a Feed Additive in Aquaculture: A Meta-Analysis

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## Abstract

Interest has recently grown in the research of humic substances (HS) as a potential aquaculture feed additive, resulting in a growing number of publications. However, previous studies have shown varying results. Therefore, to gain a comprehensive understanding of the effects of HS, a quantitative analysis conducted through a meta-analysis is required. This study employed a meta-analysis approach to evaluate the effect of HS as a feed additive on the specific growth rate (SGR) and feed conversion ratio (FCR) of fish aquaculture. The Preferred Reporting Items for Systematic and Meta-Analysis (PRISMA) method was used to collect the data. Multiple databases were searched to identify relevant articles from scholarly journals. Ultimately, 18 publications that met the requirements were included in the analysis using OpenMEE software. The results revealed an effect size (comparing the control and the HS-supplemented groups) of 0.154 (95 % CI,  $P < 0.001$ ) for SGR. Additionally, there was a notable improvement in FCR with an effect size of -0.220 (95 % CI,  $P < 0.001$ ). However, it is important to note that the analysis showed high heterogeneity ( $I^2$  values of 98.87 % and 97.91 % for SGR and FCR, respectively). In conclusion, HS supplementation may significantly improve fish growth and reduce FCRs in aquaculture. This meta-analysis contributes valuable insight into the use of HS as a beneficial feed additive in aquaculture practices.

**Keywords:** feed conversion, fish growth, humic acid, organic acid

## Introduction

Aquaculture feeds contain a wide range of ingredients that meet the nutrient requirements of fish for growth, reproduction, and immune system maintenance. Increasing numbers of non-nutritive feed additives are used in aquatic diets to aid in nutrient ingestion, digestion, absorption, and delivery to cells. Feed additives are non-nutritive ingredients added to food formulation to alter the physical or chemical qualities of the food, enhance the productivity of aquatic animals, or improve the quality of the final product (Pohlenz and Gatlin, 2014; Encarnaç o, 2015). These additives are often incorporated to achieve and maintain optimum levels of physical and chemical features in feeds. Other examples of commercially available feed additives for aquatic animals include probiotics, prebiotics, acidifiers, and plant or animal

extracts, which can directly influence fish performance and product quality (Encarnaç o, 2015; Dawood et al., 2018). Considerable variation exists in the chemical properties of these feed additives and how they are used in commercial diet formulations for aquatic animals. For instance, pellet binders, preservatives (including antibacterial and antioxidant chemicals), and feed stimulants are some examples of commercial formulations containing additives that impact feed quality (NRC, 2011).

Humic substances (HS) are organic substances derived from the humification of organic materials, classified into three categories: humic acid (HA), fulvic acid (FA), and humin (Islam et al., 2005). Humic substances have been commonly used as a feed additive in livestock and poultry (Islam et al., 2005; Arif et al., 2019) and as a fertiliser for plant growth

(Shahryari et al., 2009), while research is currently ongoing into the utilisation of HS in aquaculture. Earlier studies demonstrated various benefits of using HS as a feed additive, including improved feed digestion, enhanced disease resistance, and the removal of toxins and heavy metals, all of which contributed to increasing the growth performance of the tested fish (Steinberg et al., 2003; Abdel-Wahab et al., 2012; Gao et al., 2017; Yilmaz et al., 2018; Jusadi et al., 2020; Rasidi et al., 2021). Nevertheless, several research studies reported a variety of results in that, while supplementing with HS improved growth performance and decreased feed conversion value, it was difficult to directly compare the results of studies due to the differences in HS dosage in feed formulation, the type and source of HS, fish species, and the media conditions for rearing of fish. Thus, while previous experiments yielded a range of results, they must be quantitatively analysed in order to integrate and examine them more comprehensively.

The utilisation of HA in aquaculture has been well reviewed in a narrative study (Coban et al., 2020). However, the use of meta-analytical methods to particularly evaluate the use of HS in aquaculture has not been extensively documented. Meta-analysis is a helpful method for extracting and integrating information from several different studies (Israel and Richter, 2011). Many previous studies have used meta-analytical methods in the context of feed aquaculture (Antony Jesu Prabhu et al., 2013; Novriadi, 2017; Hua, 2021; Liland et al., 2021; Prakoso et al., 2022). However, there have been no reports of any meta-analysis of the effect of supplementing HS in feed on the growth of fish. Therefore, it was necessary to conduct a quantitative review as well as a meta-analysis on the potential applications of HS in aquaculture. The current study thus aimed to conduct a meta-analysis to explore the effects of HS as feed additives in fish diets on the growth performance and feed conversion of fish aquaculture. It is hoped that the information presented in this research can contribute to the utilisation of HS as feed additives in aquaculture feed.

## Materials and Methods

The Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) protocol was used to select articles, in line with Moher et al. (2009). As part of the comprehensive search process, relevant articles from scientific publications were sought from a variety of web science databases, including Science Direct, Google Scholar, Pub Med, and Repository Campus. All of the articles were obtained from reputable domestic and international publications with an edition written in the language of the United Nations and were published between 1999 and 2021. The literature search used the Boolean operator with several keywords, which included "humic substance", or "humic acid" or "fulvic acid and feed additives" or

"feed supplement and fish growth".

Furthermore, a reference manager was used to search and reference the articles. The database was then extracted with criteria for the selection of articles for further study. The generated articles were evaluated to ensure they met the requirements. Duplicate and inadequately titled items were removed from the reference manager, leaving a total of 35 articles and abstracts for further selection in this step. This study followed the meta-analysis steps used by Moher et al. (2009), studies must fulfil predefined criteria in order to be included in a meta-analysis database: (1) the article must list the author, date of publication, and journal; (2) the article reports HS as treatment for feed additives in fish feed; (3) the study was randomised and included the delivery of feed with and without HA (the control group). The article provided a specific growth rate (SGR) and feed conversion ratio (FCR). Articles should also include the mean, sample size, and standard deviation. In summary, 35 studies were selected for inclusion in the database. However, 17 publications were excluded because they failed to meet the criteria, which left a total of 18 articles based on the parameters given in the inquiry. The protocol flowchart shown in Figure 1 details the selection procedure, while the studies selected for consideration in this meta-analysis are summarised in Table 1. Multi-measurement changes required unit conversion. 'Unit' represents the feed HA dosage as a percentage (%). Next, the compiled articles were entered into an Excel database. Furthermore, data analysis was performed.

The mean SGR and standard deviation (SD) from the fish control diet and the fish given the HS experimental diet were extracted or derived from each study. The following equation was used to calculate SGR:

$$SGR = \frac{\ln Wt - \ln Wo}{d} \times 100$$

where Wt = final fish weight, Wo = initial fish weight, and d = day of culture.

## Data analysis

OpenMEE software (<http://www.cebm.brown.edu/openmee/help.html>) was used to perform the meta-analysis. A continuous random-effects model was employed to conduct the statistical analysis of the data. DerSimonian and Laird are two frequently used estimators. The effect size was determined using the response ratio (Hedges et al., 1999):

$$\ln r = \ln SGR_e - \ln SGR_c$$

where Ln r denotes the effect size natural log response ratio, and SGR<sub>e</sub> and SGR<sub>c</sub> represent the informed assessment of the SGRs produced by the experimental

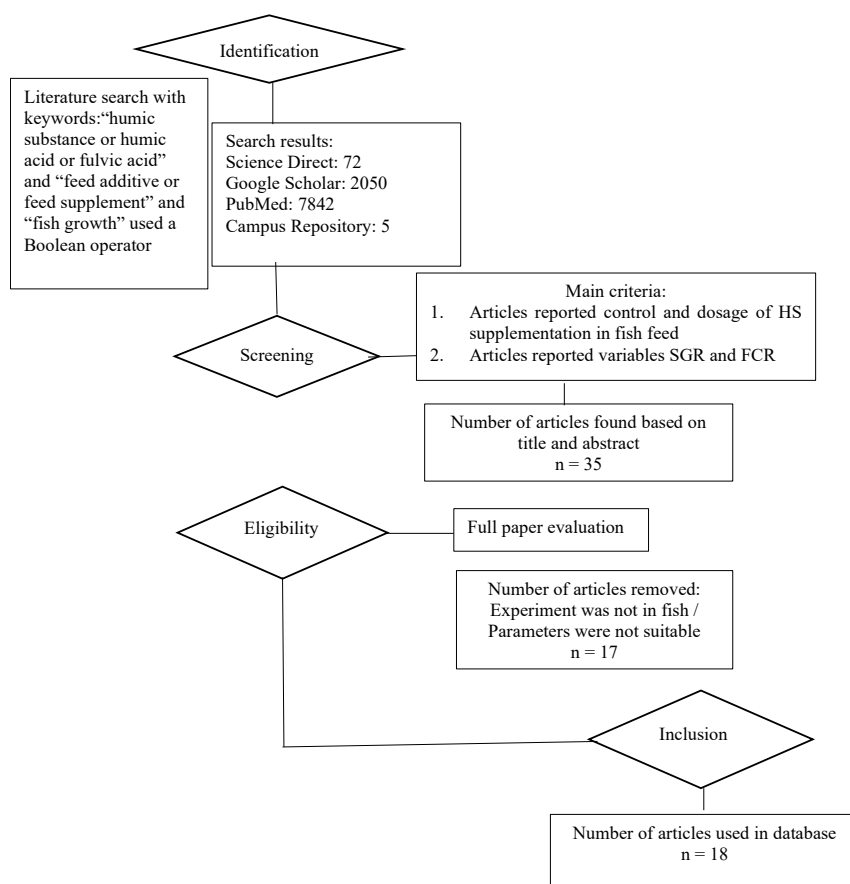


Fig. 1. Summary of flow chart process of literature selection protocol according to PRISMA (Moher et al., 2009).

Table 1. Source of data in the meta-analysis database: list of the authors, fish species, types, and humic substance dosage.

No.	Author	Common name	Species	Humic substance (HS)	Dosage (%)
1	Abdel-Wahab et al. (2012)	Common carp	<i>Cyprinus carpio</i> Linnaeus, 1758	Humic acid	0.4-1.0
2	Abdel-Hakim et al. (2014)	Tilapia	<i>Oreochromis niloticus</i> (Linnaeus, 1758)	Humic acid	0.15-0.30
3	Fahimudin (2017)	Catfish	<i>Clarias</i> sp.	Humic acid	0.05-0.2
4	Gao et al. (2017)	Loach fish	<i>Paramisgurnus dabryanus</i> Dabry de Thiersant, 1872	Fulvic acid	0.5-2.0
5	Jusadi et al. (2020)	Tilapia	<i>Oreochromis niloticus</i> (Linnaeus, 1758)	Fulvic acid	0.1-0.8
6	Deng et al. (2020)	Tilapia	<i>Oreochromis niloticus</i>	Humic acid	0.1- 0.6
7	Mandasari (2016)	Tilapia	<i>Oreochromis niloticus</i>	Humic acid	0.005-0.04
8	Marlinda (2016)	Tilapia	<i>Oreochromis niloticus</i>	Humic acid	0.01-0.08
9	Musthafa et al. (2018)	Mozambique	<i>Oreochromis mossambicus</i> (Peters, 1852)	Humic substance	2-6
10	Prokešová et al. (2021)	African catfish	<i>Clarias gariepinus</i> (Burchell, 1822)	Humic substance	1-6
11	Rasidi et al. (2019)	Asian seabass	<i>Lates calcarifer</i> (Bloch, 1790)	Humic acid	0.04-0.16
12	Rasidi et al. (2020)	Asian seabass	<i>Lates calcarifer</i>	Humic acid	0.16-2.00
13	Rasidi et al. (2021)	Asian seabass	<i>Lates calcarifer</i>	Humic acid	0.08-0.24
14	Sharaf and Tag (2011)	Common carp	<i>Cyprinus carpio</i>	Humic acid	0.02-0.04
15	Turan and Turgut (2020)	Goldfish	<i>Carassius auratus</i> (Linnaeus, 1758)	Humic substance	0.25-0.5
16	Thoriq Al Islam et al. (2021)	Catfish	<i>Clarias</i> sp.	Humic acid	0.5-2.0
17	Yilmaz et al. (2018)	Rainbow trout	<i>Oncorhynchus mykiss</i> (Walbaum, 1792)	Humic acid	0.3- 1.2
18	Yilmaz et al. (2018)	Rainbow trout	<i>Oncorhynchus mykiss</i>	Humic substance	0.10-0.40

diet and the control diet, respectively. The response ratio  $r$ , also known as the ratio of parameters in the experimental and control groups, provides an estimate of the proportional change that resulted from an experiment. The response ratio  $r$  was used to express treatment comparisons in the meta-analysis dataset (Hedges et al., 1999). The natural log-transformation of the response ratio  $r$ , the ratio of the mean SGR of fish fed a diet supplemented with HS to the mean SGR of fish fed treatment and control diets, was used to calculate the effect sizes for the meta-analysis, according to Hua (2021). The variance of  $\ln r$  was calculated as:

$$\text{Var Ln } r = \frac{(\text{SDe})^2 + (\text{SDc})^2}{n_e \text{ SGR}_e^2 + n_c \text{ SGR}_c^2}$$

where SDe and SDc are the SD of the experimental diet and the control diet, respectively, and  $n_e$  and  $n_c$  are the number of replications.

Meta-regression HS addition dosage were used to determine the study-level covariates responsible for heterogeneity. From this regression, we were able to assess the relationship between the moderators and the pooled effect size. This meta-analysis used a random-effects model, which enabled the true effect sizes to vary between the studies under consideration (Borenstein et al., 2009).

## Results

Figure 1 illustrates the literature selection process used to identify the studies that were used in the meta-analysis. A total of 18 articles were identified from the search, all of which satisfied the criteria employed in this study (Table 1). After compiling the 18 articles into a database, 42 sets of data, which will be referred to as "studies", were successfully extracted (Table 1).

The HS used comprised HA, FA, and a combination of both. Compared to other forms of feed additives, the majority of the studies (23/42 = 54.76 %) focused on the performance

of HA in fish fed 6 % of the total weight of the feed. The response ratios of the SGRs from the HS supplementation and control diets ranged from 0.18 to 0.26.

A variety of fish species were tested in the experiments, including tilapia, Asian seabass, catfish, rainbow trout, and others (Table 1). Tilapia was the most commonly used fish in the experiments, followed by Asian seabass and rainbow trout.

Protein requirements were adjusted based on the life stage of an aquatic organism as well as the species. Carnivorous fish, which are commonly found in fish marine culture, have higher recommended intakes (38–55 % protein in the diet) than omnivorous fish (20–40 %) with regard to protein consumption (NRC, 2011). The diets analysed for this meta-analysis met the protein consumption requirements of the cultured fish. The feed used had an average crude protein content of  $37.20 \pm 8.64$  %, while the crude lipid content was  $8.91 \pm 4.74$  % (Table 2). The days of culture of the test fish ranged from 17.56 to 40 days.

Forest plots are used to display the effect sizes and confidence ranges from multiple studies. The effect sizes thus cover a broad range. Overall, the fish evaluated in the experiment had SGRs and FCR after being exposed to all of the HS investigated (Figs. 2 and 3). The meta-analysis showed a mean effect size of 0.154 (95 % CI) for SGR, as indicated by the data in the SGR forest plot (Fig. 1). This results in a mean growth response of 154 % in fish that were given the HS supplementation diet.

The meta-analysis showed a statistically significant mean effect size of  $-0.220$  ( $P < 0.001$ ) for FCR (Fig. 3). The effect size value was negative, which indicates that the addition of HS in the test feed was successful in reducing the feed conversion response by 220 % to a lower overall average value.

The degree of heterogeneity in a meta-analysis can be determined by looking at the heterogeneity value ( $I^2$ ).

Table 2. The variables and summary statistics extracted from the meta-analysis database.

Variable	Unit	n	Mean	SD	Min	Max
Nutrient composition						
Crude protein	%	48	37.20	8.64	25.00	48.99
Crude lipid	%	44	8.91	4.74	4.38	19.50
Humic substance dosage	%	48	0.78	1.38	0.01	6.00
In vivo studies						
Day of culture (DOC)	day	48	64.33	17.56	40.00	140.00
Feed intake	g fish <sup>-1</sup>	25	44.90	23.36	10.59	69.90
Total digestibility	%	14	73.96	10.28	53.60	84.85
Protein digestibility	%	14	88.91	4.36	78.90	93.57
Protein retention	%	19	22.21	7.08	11.94	37.8
Specific growth rate (SGR)	% day <sup>-1</sup>	42	1.91	0.78	0.68	3.52
Feed conversion ratio (FCR)		52	1.59	0.49	0.64	2.82

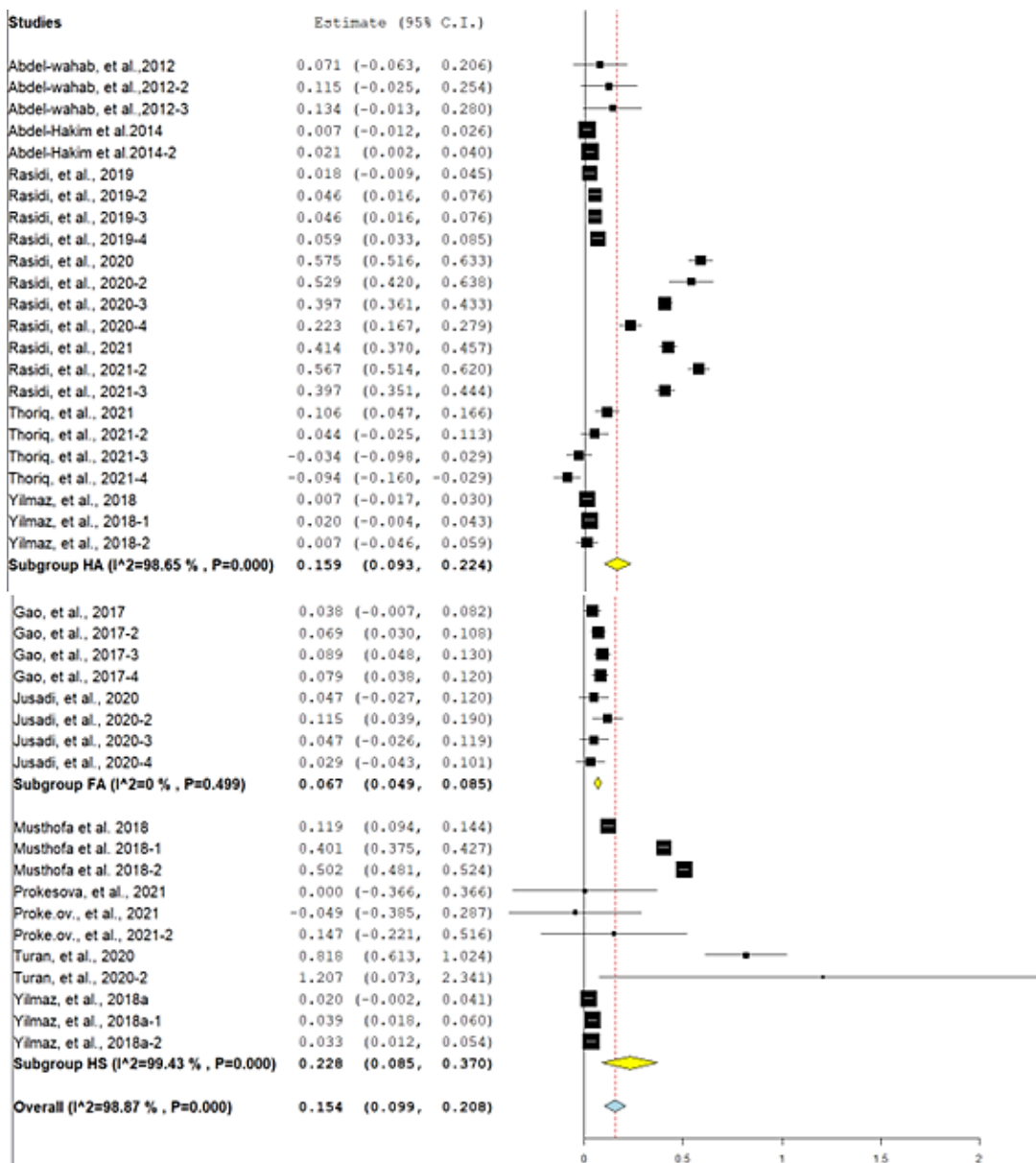


Fig. 2. Forest plot of effect sizes (Ln response ratio) experimental diets containing humic substances for the specific growth rate (SGR) parameter. The results of the SGR parameter meta-analysis presented in the form of a forest plot show the odds ratio of each study (black box) with 95 % confidence interval (CI) with overall effect size of 0.154 (0.099, -0.208),  $P = 0.000$ , which means there is significant difference between the intervention and the control group.

The heterogeneity values, given as 25, 50, and 75 %, can be thought of as low, medium, and high, respectively (Higgins et al. 2003). The  $I^2$  values for this meta-analysis were calculated to be 98.87 % ( $P < 0.001$ ) for SGR and 97.91 % ( $P < 0.001$ ) for FCR, indicating significant heterogeneity among studies. The level of heterogeneity for both parameters is therefore classified as high.

In terms of experimental diets, HA, FA, and HS – a combination of FA and HA – were considered in the articles and utilised in this study. A meta-analytical subgroup study was conducted to determine the effect of each of these categories (i.e., FA, HA or HS) on the SGR and FCR parameters. Further, the results from this meta-analytical subgroup showed that the

covariate of HS category, had a significant effect ( $P < 0.001$ ) on the effect size.

The study also indicates the potential to investigate moderator variables that influence the SGR and FCR of test fish fed with feed containing HS supplementation, particularly in comparison to control fish that were not given the same treatment. Concerning the results of the SGR and FCR meta-regression (Figs. 4 and 5), the circle illustrates the ordinate point (the size of the effect that was observed), whereas the straight line demonstrates the predictive value. The SGR regression model  $Y = 0.135 + 0.02x$  ( $R^2 = 0.042$  %,  $P < 0.001$ ) demonstrates that the SGR was significant and would increase by 0.02 % for each one (1) percentage point added to it. According to this study, the addition

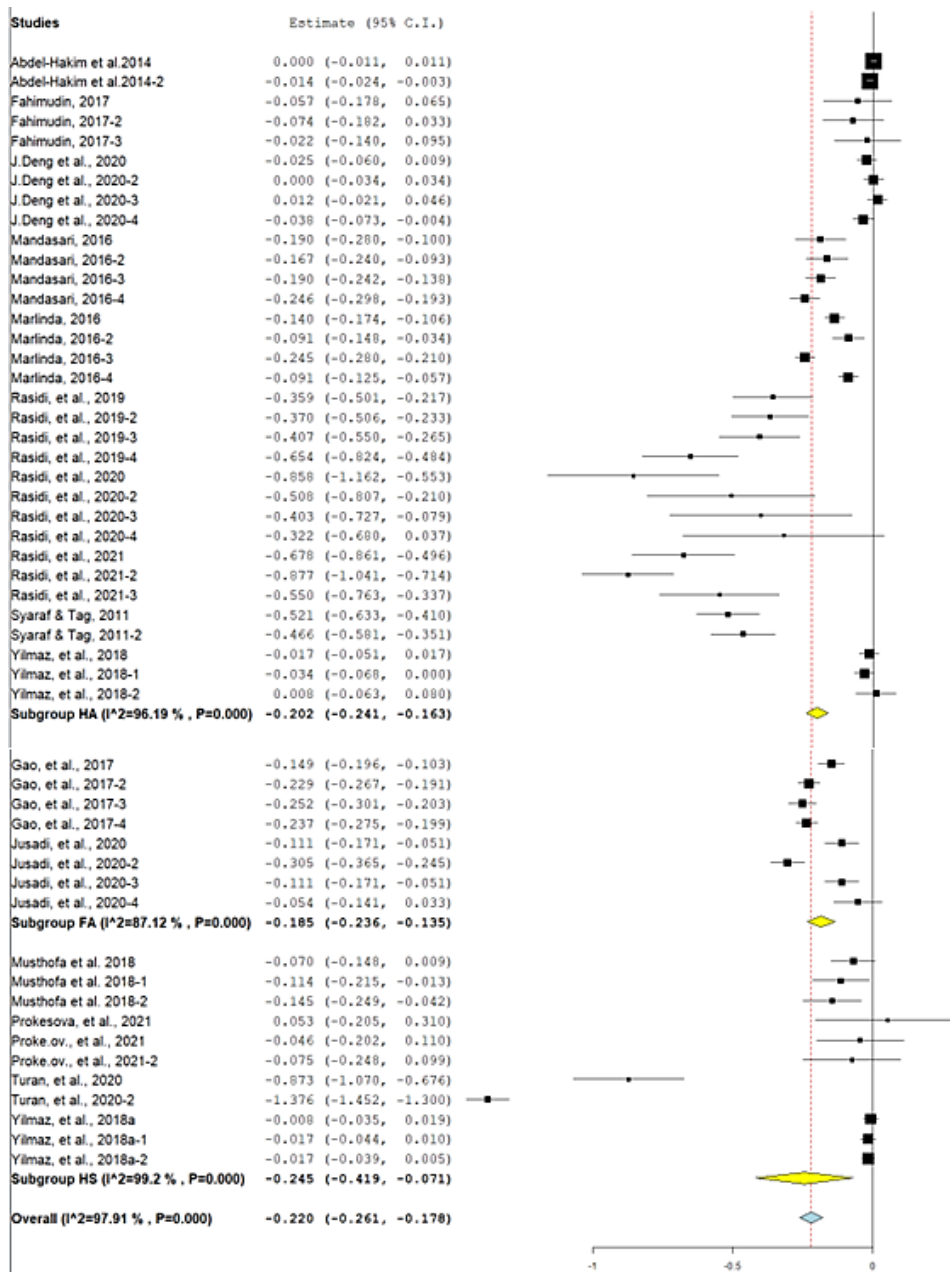


Fig. 3. Forest plot of effect sizes (Ln response ratio) experimental diets containing humic substances for the feed conversion ratio (FCR) parameter. The results of the FCR parameter meta-analysis presented in the form of a forest plot show the odds ratio of each study (black box) with 95 % confidence interval (CI) with overall effect size of  $-0.220$  ( $-0.261, -0.170$ ),  $P = 0.000$ , which means there is significant difference between the intervention and the control group.

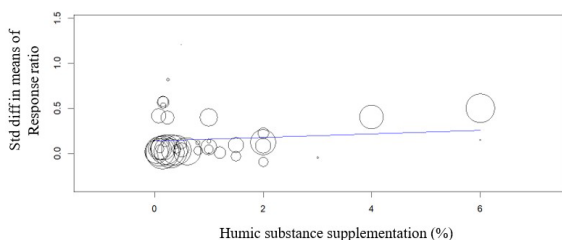


Fig. 4. The specific growth rate (SGR) regression model  $Y = 0.135 + 0.02x$ , ( $R^2 = 0.042 \%$ ,  $P < 0.001$ ,  $n = 42$ ). This means that there is strong evidence to suggest a linear relationship between response ratio of SGR and humic substances (HS) supplementation, and the coefficient  $0.02$  represents the average change in response ratio for a one-unit increase in HS supplementation.

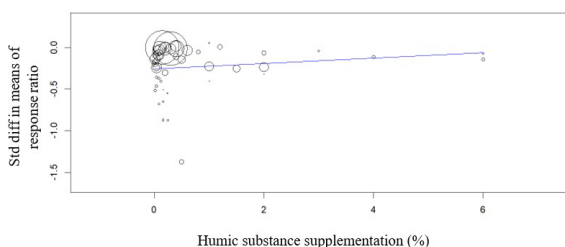


Fig. 5. The feed conversion ratio (FCR) regression model  $Y = -0.26 + 0.03x$ , ( $R^2 = 0.09 \%$ ,  $P < 0.001$ ,  $n = 52$ ). This means that there is strong evidence to suggest a linear relationship between response ratio of FCR and humic substances (HS) supplementation, and the coefficient  $0.03$  represents the average change in response ratio for a one-unit increase in HS supplementation.

of HS at concentrations of 3 % will produce optimal SGR values. The data for FCR,  $Y = -0.26 + 0.03x$  ( $R^2 = 0.09$  %,  $P < 0.001$ ), demonstrate that the FCR was significant and would reduce by 0.03 % for each one (1) percentage point supplemented to it.

## Discussion

It is clear that the studies and research on HS in aquaculture are evolving in an interesting direction, as demonstrated by the growing volume of research conducted on the topic. The research findings presented in the previous literature on the use of HS as a feed additive for aquaculture indicate that while certain variations showed positive results, the opposite also applies as other results demonstrated a negative impact from the addition of HS. The findings of the current study revealed that the response ratios varied, with an average ( $\pm$ SE) of  $0.18 \pm 0.26$  for SGR and  $-0.28 \pm 0.13$  for FCR, showing a positive effect of applying HS as a feed additive in aquaculture. The dataset in this meta-analysis showed a response ratio below 1, which indicates that the growth performance of fish fed a diet containing HS supplementation was better supported compared to that of the fish fed a control diet in the studies. This result aligns with a previous meta-analysis study in which the response ratio was below 1 (Hua, 2021).

This meta-analysis of the full dataset was indicative of the mean effect of HS on growth performance and confirmed the potency of using HS as a feed additive in fish aquaculture. The findings of the meta-analysis revealed that the inclusion of HS as a feed additive had a beneficial impact on both the growth rate and the amount of feed that was converted. This may be influenced by many factors, including the dose, type, and source of HS used in the study, thus resulting in the average effect size of adding HS to feed. For this reason, it was essential to perform a subgroup analysis to establish the effect of each covariate. The findings of this analysis demonstrated that the addition of HA, FA, and HS to fish feed produced a significant effect that was also statistically significant.

The meta-analysis showed a mean effect size of 0.154 (95 % CI: 0.099 – 0.20,  $P < 0.001$ ) for SGR and  $-0.220$  (95 % CI:  $-0.261$ – $0.178$ ,  $P < 0.001$ ) for FCR. The wide range of response ratios seen in this study reflects the varying degrees of success obtained when using HS as feed additives. Fish fed a diet supplemented with HA had much better growth performance than control fish, as indicated by the meta-analysis showing a significantly different mean effect size of zero and mean growth response. Compared with the results of previous meta-analysis studies, the findings of this study align with the mean result of the effect size response ratio having a positive impact on the growth of the test fish (Hua, 2021), and the potential for the utilisation of HS in plants to improve fish growth (Rose et al., 2014).

Based on the forest plots, it is clear that the potential influencing factors, such as the type of HS, the level of supplementation, and the balance of feed nutrition were averaged out in the meta-analysis of the complete dataset, which showed the average effect of HS as a feed additive on the growth performance of fish in the literature and confirmed the usefulness of HS in fish feed. We thus conducted a subgroup analysis to characterise the impact of these confounding factors. The extent to which HS is accounted for is correlated with the size of the physiological response to the intervention being studied. Furthermore, determining the most effective improvement rates of HS in real-world feed formulations can benefit from the establishment of correlations such as dose-response relationships or the identification of thresholds at which negative impacts are likely to occur.

According to the findings of this meta-analysis, the utilisation of HS as a feed additive has the potential to enhance the growth performance of cultured fish, particularly during the growth phase. This may be linked to the role played by the composition of HS as well as to the protein content that was present in the feed utilised. The types of HS used in this meta-analysis comprised FA, HA, and a mixture of both. Table 2 displays the summarised feed inputs that were utilised in the meta-analysis. Previous research has demonstrated the beneficial effects of providing cultured fish with a functional diet high in HA (Abdel-Wahab et al., 2012; Gao et al., 2017).

There are several mechanisms by which HAs boost growth. First, HA is a hydrophilic colloid, which means it has a high affinity for water. HA has ions functional group such as  $-\text{COOH}$ ,  $-\text{OH}$ , however, minerals and heavy metals such as  $\text{Ca}^{2+}$  and  $\text{Pb}^{2+}$  can neutralize them (Gaffney et al., 1996). Since the phenol chains and aromatic groups of carbon in HAs can attach to the atomic groups of metals, they were used as chelating agents for both heavy and essential metals. Concerning heavy metals, HS was used as a proactive measure to reduce the accumulation of heavy metals in living organisms (Flora and Pachauri, 2010). The role of functional groups that work as ion donors to heavy metal ion groups that operate as ion acceptors is indicative of the process by which HA binds heavy metals (Farouk et al., 2011). This results in chelation and complex interactions between HA ions and heavy metals. When heavy metals are bound, they lose their ability to react, which prevents them from accumulating in the organs and makes it simpler for the body to release them. According to Trckova et al. (2005), HA can operate as a chelator, which means that it can bind essential mineral metals and hazardous compounds, including heavy metals. Studies conducted on a variety of fish have regularly shown that the addition of HA had a beneficial effect on reducing heavy metals accumulation and increasing the growth and performance of tilapia (Marlinda, 2016; Jusadi et al.,

2020) and Asian seabass (Rasidi et al., 2020, 2021).

There have been reports on several different functional classes of HS (Islam et al., 2005). According to the findings of research by Prokešová et al. (2021), HS, including phenolic functional groups and numerous essential minerals (such as Ca and Fe), could increase fish growth performance when supplemented in catfish diets. In addition, Peña-Méndez et al. (2005) reported that HS contain functional groups such as phenols and trace minerals, both of which contribute to an improvement in the overall health condition and growth of the animals. These mechanisms include the role that HAs play in regulating the microbiota in the digestive tract, increasing enzyme secretion, digestion, and nutrient absorption, and improving immune function (Mandasari, 2016; Gao et al., 2017).

The HS used comprised HA and FA, while the proportions included in the feed formulation ranged from 0.02 % to 6 %. Feed additives derived from a wide variety of sources have been extensively researched and implemented in aquaculture. With the increase in the number of studies published on the effects of HS on various types of fish, it became clear that HS was attracting interest and should be explored.

HAs decrease pathogen growth by regulating the pH in the digestive system, blocking virus particle adhesion to cells through antiviral action, increasing calcium and trace element utilisation, and minimising heavy metal toxicity (Tyler and McBride, 1982; Spark et al., 1997). HAs are strong antioxidants that scavenge free radicals (Ipek et al., 2008). HS could help stabilise gut flora, thus promoting efficient nutrient utilisation in animal feed (El-Husseiny et al., 2008; Gao et al., 2017). HAs form part of the natural environment of fish and thus offer potential as an environmentally friendly means of reducing disease pathogens (Lieke et al., 2020).

The subgroup analysis also examined the influence of different kinds of HS on fish growth. When comparing the growth rates of fish fed the experimental and control diets, it was revealed that high HS inclusion rates (over 3 %) inhibited fish growth. When determining the efficacy of HS supplementation as a feed additive, the results demonstrate the importance of ensuring the nutritional similarity of experimental diets.

In terms of research considering FA and HS, the use of HS in aquaculture feed has, to date, been the major focus of HA research. Due to the higher weight of the HA data set relative to those for FA and HS, it is highly likely that the HA data set significantly impacts the findings from the full data set. Given this limitation, it is important to exercise caution regarding the findings of meta-analyses based on comprehensive data sets. The overall results involving the various kinds of HS were more informative than the subgroup analyses.

Given the divergent effects of FA and HS, it is crucial to determine the optimum doses for each kind of HS. In this study, the HS supplementation rate varied: the inclusion rate of HA in the experimental diet was between 0.5 and 6 %, while that of FA was between 0.3 and 6 %. The difference in the impact between the two forms of HS was thus probably an artefact of the varied level of HS inclusion under study and a reflection of the genuine difference between the quality of the sources of the types of HS used (Rose et al., 2014).

## Conclusion

This meta-analysis concludes that HS supplementation in feed can improve fish growth and reduce FCR. A comparison of the data on the size of the impact of HA supplementation dosage between the control condition and the diet formulation showed 0.154 for SGR and -0.220 for FCR. The confidence intervals are shown at 95 %. The meta-regression study revealed that HS supplementation improves fish SGR and reduces feed conversion. Furthermore, based on the database in this study, HS would be a good feed additive for practical diets.

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**Author contributions:** Rasidi Rasidi: Conceptualisation, methodology, writing-original draft. Idil Ardi: Collected, compiled, and analysed data. Dewi Puspaningsih: Collected, compiled, and analysed data. Vitas Atmadi Prakoso: Data analysis and review. Dedi Jusadi: Writing-review and supervision. Anuraga Jayanegara: Writing-review and supervision.

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