

The Effect of *Schizochytrium* sp. on the Growth and Health of Fish

ABSTRACT

The rapidly expanding aquaculture sector of the worldwide fisheries industry is mostly due to fish feed. Omega-3 long-chain polyunsaturated fatty acids, which are good for fish health and growth, are mostly found in fish oil. However, the cost of fish oil is increasing while its production is declining. Aquaculture can be maintained with the use of alternate aquafeeds. Due to their quick growth, aquatic protists have a lot of potential as a source of omega-3 oil. *Schizochytrium* sp. can be used to substitute fish oil in aquafeed due to its high lipid and DHA content, according to numerous research. The purpose of this review is to determine the effects of utilizing *Schizochytrium* sp. in fish feed on fish growth and health. The nutritional value and optimal dosage of *Schizochytrium* sp. for various fish sizes are discussed. The optimal concentration of *Schizochytrium* sp. varies by species and can replace fish oil in the range of 20–80%. Several studies found that replacing fish oil in fish food with *Schizochytrium* sp. made the fish grow faster, live longer, gain weight, and eat more food. In the future, *Schizochytrium* sp. and several types of marine protists have the potential to serve as alternative sources that can replace fish oil in fish feed.

Keywords: Aquafeed; *Schizochytrium* sp.; fish oil; DHA; protists.

1. INTRODUCTION

Aquaculture is one of the sectors that can meet human food needs. Based on data from FAO [1], total aquaculture production in the world reached 122.6 million tonnes of the 214 million tonnes of total fisheries and aquaculture in 2020. This value shows that the contribution of aquaculture to the fisheries sector is equivalent to 49.2%. The total production came from inland waters (54.4 million tonnes) and marine aquaculture (68.1 million tonnes). In 2019, the global annual per capita consumption of aquatic foods will reach 20.5 kg. In several countries, such as Indonesia, Ghana, Cambodia, and Bangladesh, aquatic foods meet protein intake needs of around 50% or more. Meanwhile, globally, aquatic food fulfils 20% of the average per capita intake of animal protein. This shows that aquaculture is a sector that has the potential to continue to be developed.

One of the factors that influences the success of aquaculture is the availability of quality feed that meets the nutritional needs of fish. N-3 highly unsaturated fatty acids (n-3 HUFA) such as docosahexaenoic acid (DHA) are important dietary lipids for growth, fish reproductive processes [2], lipid metabolism, fish immunity [3], membrane permeability and plasticity, enzyme

activation, prostaglandin production [4], and stress tolerance increase [5]. The ability of freshwater fish to modify C18 to produce Hufa is well documented [6]. However, in general, marine fish cannot synthesize Hufa, so it needs to be available in fish feed [7]. Fish oil is usually used as a source of fatty acids, but the price of fish oil is increasing and its production is decreasing [8]. This will lead to an increase in production costs [9]. Meanwhile, the demand for fish oil continues to increase for the aquaculture sector so that it can reduce fish oil intake for other sectors such as food and medicine [10]. Fish oil is not a sustainable source of omega-3 oil. On the other hand, aquatic protists have a lot of potential as a source of omega-3 oil [11]. For this reason, a lot of research is being done to find new alternative sources of omega-3 from aquatic protists.

Schizochytrium sp. is a fast-growing thraustochytrid, containing 66% lipid with high DHA [12]. Depending on the cultivation method, lipid and DHA levels in *Schizochytrium* sp. can be increased. DHA and EPA content in *Schizochytrium* sp. can be increased to 81.5% and 172.5%, respectively [11]. Several researchers have reported the effect of *Schizochytrium* sp. supplementation in fish feed as a source of DHA. Furthermore, by using food

waste as a nutrient source, the production costs of *Schizochytrium* sp. (also known as *Aurantiochytrium* sp.) as a source of DHA can be reduced by up to 35% [13]. This review article aims to describe the effect of *Schizochytrium* sp. supplementation on fish feed and its potential to be used as a fish oil substitute.

2. *Schizochytrium* sp.

Schizochytrium sp. is a spherical, unicellular microorganism that lives in seawater [14], freshwater [12] or brackish water. Protist with a cell diameter of 9–14 µM can form biflagellate zoospores, aplanospores, and amoeboid cells. Species in the genus *Schizochytrium* are distinguished based on the formation, size, and number of zoospores present in the zoosporangium [15]. *Schizochytrium* sp. reproduces by means of cell division. The availability of oxygen in the water is important for the development of this aerobic heterotrophic protist [16]. *Schizochytrium* sp. classification is as follows:

Kingdom:	Chromista
Phylum:	Bigyra
Class:	Labyrinthula
Order:	Thraustochytriida
Family:	Thraustochytriaceae
Genus:	<i>Schizochytrium</i>

[17]

Additionally, disputes about the taxonomic classification of the genus *Schizochytrium* have been going on 2007. Changes to the classification were suggested by Yokohama and Honda [10] based on genetic and phenotypic data. New genera were defined, including *Aurantiochytrium* and *Oblongichytrium*, and the name *Schizochytrium* was modified. As a result, *Aurantiochytrium* can now be used to refer to the genus *Schizochytrium* [17].

3. NUTRITIONAL VALUE OF *Schizochytrium* sp.

Schizochytrium sp. contains various nutrients that are good for fish growth, including protein, carbohydrates, lipids, fiber [18], and vitamins. The vitamins contained include biotin (0.3 mg), choline (1440 mg), folic acid (0.1 mg), niacin (14 mg), vitamin a (33.6 µg), beta carotene (2.3 µg), vitamin b1 (4.4 mg), vitamin b2 (2.9 mg), vitamin b6 (1.4 mg), vitamin b12 (54.9 µg), vitamin e (0.45 µg), and pantothenic acid 3.5 mg [18]. The minerals contained include magnesium, calcium, sodium, and other metal elements [19]. SFA

(saturated fatty acid) (233 g/kg), MFA (mono-unsaturated fatty acids) (53 g/kg), and PUFA (poly unsaturated acid) (713 g/kg) are all present in this protist (Sharker et al., 2015). The content of DHA in dried *Schizochytrium* sp. is around 35% DHA of total fatty acids [20]. In addition, *Schizochytrium* sp. contains several bioactive compounds, such as flavonoids, β-glucans, β-carotene, polysaccharides, nucleotides, and peptides. *Schizochytrium* sp. contains amino acids that are good enough for digestion, including arginine, lysine, isoleucine, histidine, methionine, threonine, tryptophan, and valine [18].

4. THE EFFECT OF *Schizochytrium* sp. ON FISH GROWTH

Several studies have shown that *Schizochytrium* sp. can be used as an alternative ingredient to replace fish oil in various types of fish, either partially or completely. This protist can also be used as a feed additive. *Schizochytrium* sp. can replace 20%–80% of the fish oil in juvenile rainbow trout feeds [8]. Replacement of 20% fish oil by *Schizochytrium* sp. showed growth performance (weight gain, SGR, FE, and PER), which was greater than the control (100% fish oil) and other treatments (40, 60, 80, and 100% *Schizochytrium* sp.). The nutrient composition of the fish's body was also not significantly different from the control. Another study on juvenile rainbow trout was conducted by Osmond et al [21], who tested the replacement of fish oil (FO) as a whole with *Schizochytrium* sp. oil (MO) or with Camelina oil and *Schizochytrium* sp. (MO/CO) oil. The results showed that the overall replacement of fish oil by *Schizochytrium* sp. showed growth performance similar to that of the control (fish oil).

Substitution of fish oil with *Schizochytrium* sp. can be done on gilthead seabream fish (*Sparus aurata*). Santigosa et al [22] tested the use of *Schizochytrium* sp. Oil, rapeseed oil, and camelina oil to completely replace fish oil. The results showed that the replacement of fish oil with these three oils did not make a significant difference in the growth performance of gilthead seabream (*Sparus aurata*). This demonstrates the ability of *Schizochytrium* sp. to replace fish oil. Karapanagiotidis et al [23] also tested the effect of replacing 50% and 100% of the fish oil with *Schizochytrium* sp. and *Microchloropsis* sp. on gilthead seabream (*Sparus aurata*) feed. The test results showed that the weight gain and specific growth rate of gilthead seabream (*Sparus aurata*) were higher than the control.

Table 1. Nutritional value of *Schizochytrium* sp.

Nutrient	Value	References
Basic Components		
Protein	12.1%	[18]
Carbohydrates	32.0%	
Crude fat	45.3%	
Fiber	0.6%	
Fatty Acid		
SFA (saturated fatty acid)	233 g/kg	Sharker et al., 2015
MFA (mono-unsaturated fatty acids)	53 g/kg	
PUFA (poly unsaturated acid)	713 g/kg	
Vitamins		
Biotin	0.3 mg	[18]
Choline	1400 mg	
Folic acid	0.1 mg	
niacin	14 mg	
Vitamin a	33.6 µg	
beta carotene	2.3 µg	
Vitamin b1	4.4 mg	
Vitamin b2	2.9 mg	
Vitamin b6	1.4 mg	
Vitamin b12	54.9 µg	
Vitamin e	0.45 µg	
Pantothenic acid	3.5 mg	

Apart from rainbow trout and gilthead seabream *Sparus aurata*, partial or complete replacement of fish oil with *Schizochytrium* sp. is also performed on juvenile Nile tilapia (*Oreochromis niloticus*). Weight gain and protein efficiency ratio in fish fed artificial feed with 100% replacement fish oil from *Schizochytrium* sp. were higher than control [24]. The addition of *Schizochytrium* sp. on artificial feed at various concentrations of 10, 20, 30, and 40% can increase weight gain, biomass, specific growth rate, and feed intake of juvenile Piau (*Leporinus friderici*). Fish fed artificial feed supplemented with 40% *Schizochytrium* sp. showed the best growth performance [25]. This is presumably because the *Schizochytrium* sp. can be digested properly by fish [24]. The main reason is that it has a lot of DHA, which is an important molecule for fish to grow [26].

5. EFFECT OF *Schizochytrium* sp. ON FISH HEALTH

Schizochytrium sp. can be used as a substitute for fish oil or as an additive in feed because it has a good effect on fish health. Zebrafish fed with the addition of 120 g/kg *Schizochytrium* sp. showed an increase in the survival rate and density of goblet cells in the intestinal wall when challenged with *Edwardsiella piscicida* [27]. Ibrahim et al. [28] conducted a feed test

containing a mixture of *Nannochloropsis oculata* and *Schizochytrium* and *Spirulina* species at equal proportions (1:1:1) for 12 weeks with various added concentrations (0.75, 1.5, and 3%) to Nile tilapia (*Oreochromis niloticus*). Giving a mixture of the three microorganisms is known to significantly increase serum lysozyme activity. Nitric oxide (NO) and alternative complement pathway activity (ACH50) increased as the concentration of the microorganisms mixture increased.

Substitution of fish oil with various concentrations of *Schizochytrium* sp. i.e., 20, 40, 60, 80, and 100% substitution, is known to have a positive effect on the health of rainbow trout. This was indicated by the fact that the lysozyme activity of rainbow trout fed 20% *Schizochytrium* sp. was the highest, while the activity of lysozyme in the control and other treatments was not significantly different. An increase in lysozyme activity indicates an increase in the immune response of fish. When fish were challenged with *Lactococcus garvieae*, 20% *Schizochytrium* sp. significantly increased their survival rate [8].

The addition of 3% *Schizochytrium* sp. on feed of golden pompano (*Trachinotus ovatus*) for 8 weeks can increase the survival rate (92.50%) compared to control diet (76.25%). In the diet treatment group supplemented with *Schizochytrium* sp., gut amylase and lipase

levels were considerably higher than in the control group (P 0.05). In the *Schizochytrium* sp supplemented treatment group compared to the control group, the relative level of peroxisome proliferator-activated receptor (PPAR) expression in the liver was considerably increased (P 0.05). The addition of 3% *Schizochytrium* sp. on feed of golden pompano (*Trachinotus ovatus*) for 8 weeks can increase non-specific immunity [29].

6. CONCLUSION

Application of *Schizochytrium* sp. as a substitute for fish oil in feed ingredients improved growth performance, survival rate, and health of fish. The amount of *Schizochytrium* sp. supplemented to feed varies depending on the type of fish. The high nutritional value of *Schizochytrium* sp. can help to support the use of alternative sources, as a substitute for fish oil in feed ingredients, to help support the sustainability of aquaculture.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. FAO. The State Of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome, FAO; 2022. Available:<https://doi.org/10.4060/cc0461en>
2. Furuita H, Tanaka H, Yamamoto Y, Suzuki N, Takeuchi T. Effects of high levels of N-3 HUFA in broodstock diet on egg quality and egg fatty acid composition of Japanese flounder, *Paralichthys olivaceus*. *Aquaculture*. 2002;210:323-333.
3. Li M, Xu C, Ma Y, Ye R, Chen H, Xie D, Zhang G, Zhang M, Wang M, You C, Wang S, Ning L, Luo M, Li Y. Effects of dietary N-3 highly unsaturated fatty acids levels on growth, lipid metabolism and innate immunity in juvenile golden pompano (*Trachinotus ovatus*). *Fish & Shellfish Immunology*. 2020;105:177–185. Available:<https://doi.org/10.1016/j.fsi.2020.06.060>
4. Dadgar S. Effect of different levels of N-3 Hufa on larvae culture performances of beluga (*Huso huso*) fish. *Iranian Journal of Fisheries Sciences*. 2016;15(2):751–759.
5. Mutti DW, Ballester ELC, Martino RC, Wasielesky WJ, Cavalli RO. Feeding N-3 hufa enriched *Artemia* to the larvae of the pink shrimp *Farfantepenaeus Paulensis* increases stress tolerance and subsequent growth. *Latin American Journal of Aquatic Research*. 2017;45(1).
6. Ren HT, Zhang GQ, Huang Y, Gao XC. Effects of different dietary lipid sources on fatty acid composition and gene expression in common carp. *Czech Journal of Animal Science*. 2020;65(2):51–57.
7. Lee SM, Lee JH, Kim KD. Effect of dietary essential fatty acids on growth, body composition and blood chemistry of juvenile starry flounder (*Platichthys stellatus*). *Aquaculture*. 2003;225:269–281.
8. Lee S, Park CO, Choi W, Bae J, Kim J, Choi S, Katya K, Kim KW, Bai SC. Partial substitution of fish oil with microalgae (*Schizochytrium* sp.) can improve growth performance, nonspecific immunity and disease resistance in rainbow trout, *Oncorhynchus mykiss*. *Animals*. 2022;12(9):1220. Available:<https://doi.org/10.3390/ani12091220>
9. Medagoda N, Kim MG, Gunathilaka BE, Park SH, Lee KJ. Effect of total replacement of fish oil with tallow and emulsifier in diet on growth, feed utilization, and immunity of olive flounder (*Paralichthys olivaceus*). *Journal of The World Aquaculture Society*. 2021:1-14
10. Yokohama R and Honda D. Taxonomic rearrangement of the genus schizochytrium sensu lato based on morphology, chemotaxonomic characteristics, and 18s rRNA gene phylogeny (thraustochytriaceae, labyrinthulomycetes): Emendation for schizochytrium and erection of aurantiochytrium gen. *Mycoscience*. 2007; 48:199-211. Available:<https://www.globenewswire.com>
11. Russo GI, Langelotti AI, Oliviero M, Sacchi R, Masi P. Sustainable production of food grade omega-3 oil using aquatic protists: reliability and future horizons. *N Biotechnol*. 2021;62:32-39. DOI:10.1016/J.Nbt.2021.01.006
12. Allen KM, Habte-Tsion HM, Thompson KR, Filer K, Tidwell JH, Kumar V. Freshwater microalgae (*Schizochytrium* Sp.) as a substitute to fish oil for shrimp feed. *Sci Rep*. 2019;9:6178. Available:<https://doi.org/10.1038/s41598-019-41020-8>
13. Russo GL, Langelotti AL, Sacchi R, Masi P. Techno-economic assessment of dha-rich *Aurantiochytrium* Sp. production using

- food industry by-products and waste streams as alternative growth media. *Bioresource Technology Reports*. 2022a; 18:100997.
DOI: 10.1016/J.Biteb.2022.100997
14. Wang X, Wang H, Pierre JF, Wang S, Huang H, et al. Marine microalgae bioengineered *Schizochytrium Sp.* meal hydrolysates inhibits acute inflammation. *Scientific Reports*. 2018;8:9848.
Available: <https://doi.org/10.1038/s41598-018-28064-y>
 15. Honda D, Yokochi T, Nakahara T, Erata M, Higashihara T. *Schizochytrium Limacinum Sp. Nov.*, a new thraustochytrid from a mangrove area in the West Pacific Ocean. *Fungal Biology*. 1998;102:439-448.
 16. Bi ZQ, Ren LJ, Hu XC, Sun XM, Zhu SY, Ji XJ, Huang H. Transcriptome and gene expression analysis of docosahexaenoic acid producer *schizochytrium Sp.* under different oxygen supply conditions. *Biotechnology for Biofuels*. 2018;11:249.
Available: <https://Doi.Org/10.1186/S13068-018-1250-5>
 17. EFSA. Panel on Nutrition, Novel Foods and Food Allergens, Turck D, Castenmiller J, De Henauw S, Ildico K et al. Safety of oil from *Schizochytrium limacinum* (strain fcc-3204) for use in infant and follow-on formula as a novel food pursuant to regulation (eu) 2015/2283. *EFSA Journal*. 2021;19(1).
 18. Hadley KB, Bauer J, Milgram NW. The oil-rich alga *Schizochytrium Sp.* as a dietary source of docosahexaenoic acid improves shape discrimination learning associated with visual processing in a canine model of senescence. *Prostaglandins Leukot Essent Fatty Acids*. 2017;118:10-18.
DOI:10.1016/J.Plefa.2017.01.011
 19. Song Z, Peng YB, Song YF, Wang QK, He YH, Liu S. Analysis of nutrient composition and polysaccharide characteristics of fungus *Schizochytrium Sp.* *Journal of Dalian Ocean University*. 2019;34(2):247-251.
 20. Russo GI, Langellotti AI, Verardo V, Martín-García B, Di Pierro P, Sorrentino A, Baseliace M, Oliviero M, Sacchi R, Masi P. Formulation of new media from dairy and brewery wastes for a sustainable production of dha-rich oil by *Aurantiochytrium mangrovei*. *Marine Drugs*. 2022b;20(1):39.
Available: <https://Doi.Org/10.3390/Md20010039>
 21. Osmond ATY, Arts MT, Hall JR, Rise MI, Bazinet RP, Armenta RE, Colombo SM. *Schizochytrium Sp.* (T18) oil as a fish oil replacement in diets for juvenile rainbow trout (*Oncorhynchus mykiss*): Effects on growth performance, tissue fatty acid content, and lipid-related transcript expression. *Animals*. 2021;11(4):1185.
Available: <https://doi.org/10.3390/ani11041185>
 22. Santigosa E, Brambilla F, Milanese L. Microalgae oil as an effective alternative source of epa and dha for gilthead seabream (*Sparus Aurata*) aquaculture. *Animals*. 2021;11(4):971.
Available: <https://doi.org/10.3390/ani11040971>
 23. Karapanagiotidis IT, Metsoviti MN, Gkalogianni EZ, Psoufakis P, Asimaki A, Katsoulas N, Papapolymerou G, Zarkadas I. The effects of replacing fishmeal by *Chlorella vulgaris* and fish oil by *Schizochytrium Sp.* and microchloropsis gaditana blend on growth performance, feed efficiency, muscle fatty acid composition and liver histology of gilthead seabream (*Sparus aurata*). *Aquaculture*. 2022;561:738709.
Available: <https://doi.org/10.1016/j.aquaculture.2022.738709>.
ISSN 0044-8486
 24. Sarker PK, Kapuscinski AR, Lanois AJ, Livesey ED, Bernhard KP, Coley MI. Towards sustainable aquafeeds: Complete substitution of fish oil with marine microalga *Schizochytrium Sp.* improves growth and fatty acid deposition in juvenile Nile tilapia (*Oreochromis niloticus*). *Plos One*. 2016;11(6):E0156684.
DOI: 10.1371/Journal.Pone.0156684
 25. Prates AS, Schorer M, Moura GD, Lanna EA, Castro GH, Pedreira MM. Microalgae *Schizochytrium Sp.* in feed for piau leporinus friderici. *American Journal of Animal and Veterinary Sciences*. 2018;13:130-135.
 26. Kumar N, Chandan NK, Gupta SK, Bushan S, Patole PB. Omega-3 fatty acids effectively modulate growth performance, immune response, and disease resistance in fish against multiple stresses. *Aquaculture*. 2022;547:737506.
Available: <https://doi.org/10.1016/j.aquaculture.2021.737506>
 27. Shi Y, Cao X, Ye Z, Xu Y, Wang Y, Li Z, Hang W, He N. Role of dietary *Schizochytrium Sp.* in improving disease

- resistance of zebrafish through metabolic and microbial analysis. *Aquaculture*. 2021; 539:736631.
28. Ibrahim D, Abd El-Hamid MI, Al-Zaban MI, Elhady M, El-Azzouny MM, Elfeky TM, Al Sadik GM, Samy OM, Hamed TA, Albalwe FM, et al. Impacts of fortifying Nile tilapia (*Oreochromis Niloticus*) diet with different strains of microalgae on its performance, fillet quality and disease resistance to *Aeromonas hydrophila* considering the interplay between antioxidant and inflammatory response. *Antioxidants*. 2022;11:2181. Available: <https://doi.org/10.3390/Antiox11112181>
29. Xie J, Fang H, Liao S, Guo T, Yin P, Liu Y, Tian L, Niu J. Study on *Schizochytrium Sp.* Improving the growth performance and non-specific immunity of golden pompano (*Trachinotus ovatus*) while not affecting the antioxidant capacity. *Fish & Shellfish Immunology*. 2019;95:617–623. Available: <https://doi.org/10.1016/j.fsi.2019.10.028>

© 2022 Pratiwi and Zidni; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.