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A systematic review on the potentiality of algal meal as an aquaculture feed ingredient

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ABSTRACT

Aquaculture is a vital source of protein and polyunsaturated fatty acids for humans. However, there are many obstacles to the sustainable growth of aquaculture, such as a lack of high-quality feed and feedstock and a decline in the quality and safety of aquatic goods. This paper investigates the potential of microalgae meal alternatives to fish meal to enhance the quality and health of fish species and shrimp. The microalgae and macroalgae play an important role in recent research for the production of diverse biopolymers. English databases such as PubMed, Google Scholar and Embase were queried from 2015 to 2024. We mined a total of 221 research documents, of which 11 articles were selected based on the inclusion and exclusion criteria. Both microalgae and algal products were found to positively impact the growth, fatty acid composition, polyunsaturated fatty acid concentration, and immunity of the different varieties of fish. However, algal feed dosage varied based on geographical location, algae and fish variety. Low dosages of algal feed were more efficient than high dosages. The study concludes that an algal feed mixture can partially replace fish meal in the aquaculture industry

Keywords: Microalgae; algal meal; fish meal; fatty acid composition; growth performance

1. INTRODUCTION

Aquaculture plays a significant role in the global economy and food production. According to 2021 statistics, the aquaculture industry production of live organisms was 126 million tonnes with an estimated value of USD 296.5 billion [1]. As the global demand for nutritious protein food is increasing, the aquaculture industry is anticipated to play a potent role in meeting the global food demand. Sustainable and ethical methods are essential for the long-term sustainability of the aquaculture sector and the ecosystem [2]. Most commercial aquatic species depend on feed supplements for growth and development [3]. Aquatic feeds such as fish meal and fish oil prepared using small fishes and fish waste were traditionally used as feed mixtures. Although fish meal is a rich source of proteins, essential amino acids, and fatty acids, the demand for fish meal has rapidly increased over the past decade [4]. Wild fish, such as pelagic species, are being used for fish meal preparation. However, pelagic species are fast depleting due to overfishing and high temperatures leading to limited and unpredictable harvests [5]. Hence, current research is going on in search of sustainable and suitable alternatives to fish meal.

Many alternative protein feeds, including plant extract-based feeds (soya meal, guar meal, almond meal, moringa meal), insect meal, yeast meal, algal meal, and Spirulina meal, are currently being researched for the fish feed mixture [6, 7].

Among these, algae, including micro and macroalgae, were found to have an appropriate biomass composition, including protein, carbohydrates, and fats [8]. Owing to these composition, microalgae and macro algae are mainly used for the production of biopolymers and bioplastics. Microalgae such as Porphyridium, Chlorella, Nannochloropsis, and Chlamydomonas are rich sources of essential amino acids (such as Methionine), digestible carbohydrates, and fiber-rich complex carbohydrates [9-11]. Also, microalgae have low levels of hemicellulose and do not have lignin in their cell walls, thus making them easily digestible [12]. However, the main disadvantage is their rigid cell wall and high production costs [13].

Although a lot of research is being done on the formulation of microalgae as an aquatic feed mixture, only very few literature reviews are available to analyze the published data. Generally, microalgal based biomass is used for producing bioplastics and biopolymer and recent studies using it as feedstock for secondary processes. The biopolymers and bioplastic obtained from microalgal biomass are comparable with existing plastics. These biopolymers or bioplastics are biodegradable, which also makes them environment-friendly. Hence, the present study aims to systematically review the prospects of algal feed mixtures as an alternative to fish feed in the aquaculture industry and discuss the potential and sustainability of algal feed mixtures in the long run.

2. METHODOLOGY

2.1 Literature Search

PRISMA guidelines for systematic review and meta-analysis were followed in conducting the literature search to evaluate the potential of algae feed mixture as an alternative to fish feed in the aquaculture industry [14]. The inclusion criteria for screening of the research articles include only English language experimental studies on algal feed mix in aquaculture. The exclusive criteria include uncontrolled studies, laboratory studies, and case reports. Literature search is done only in English databases such as PubMed, Embase, and Google Scholar. The search was confined to the time period from 2000 to 2023. For Google

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Scholar, the main keywords used were algal meal, fish meal, fish meal alternatives, and aquaculture feeds. The pertinent medical subject headings (MeSH) used were ("Cyanobacteria"[Mesh]) OR ("Chrysophyta"[Mesh] OR "Prototheca"[Mesh] OR "Algal Proteins"[Mesh] OR "Kelp"[Mesh] OR "Scenedesmus"[Mesh] OR "Fucus"[Mesh] OR "Ascophyllum"[Mesh] OR "Macrocystis"[Mesh] OR "Ochromonas"[Mesh] OR "Cryptophyta"[Mesh] OR "Harmful Algal Bloom"[Mesh] OR "Stramenopiles"[Mesh] OR "Microalgae"[Mesh] OR "Chlorophyta"[Mesh] OR "Phaeophyceae"[Mesh] OR "Rhodophyta"[Mesh]) AND "Fish meal". After carefully considering all of our possibilities, we chose the study with the largest sample size or the most recent publication for our research samples. We then searched further into the publications' references to locate related studies.

2.2 Selection and Screening

The screening approach of research articles was performed separately by two researchers. In the first step, research documents were screened based on the title and abstract. The selected research documents were thoroughly reviewed based on the inclusion and exclusion criteria, and articles that did not meet the inclusion criteria were excluded from the study. The research articles were excluded if the study did not meet the necessary selection criteria. Finally, the researcher independently retrieved pertinent data from the included studies using a pre-designed data-collecting form. Any differences in the data collected were sorted out through discussion. The primary contents of the data collection form include the title of the research article, author name, year of publication, study design, algal meal components, fish meal components, fish species, meal preparation strategies, methodology, outcomes of the study, and significance or conclusion of the study.

2.3 ROB analysis

SYRCLE's risk of bias tool for animal studies has been employed [15]. The following questions were considered to determine the bias of the selected articles.

- Was the allocation sequence adequately generated and applied?
- Were the groups similar at baseline or were they adjusted for confounders in the analysis?
- Was the allocation adequately concealed?
- Were the animals randomly housed during the experiment?
- Were the caregivers and/or investigators blinded from knowledge of which intervention each animal received during the experiment?
- Were animals selected at random for outcome assessment?
- Was the outcome assessor blinded?
- Were incomplete outcome data adequately addressed?
- Are reports of the study free of selective outcome reporting?
- Was the study free of other problems that could result in a high risk of bias?

All the questions were answered with Yes, No, and Unclear. "Yes" indicated low risk of bias, "No" indicated poor quality or particular criteria were not fulfilled, and high risk of bias (Table 1 suppl)

3. RESULTS

3.1 Literature search and study characteristics

Figure 1 displays a Prisma flowchart summarizing the systemic review search approach and inclusion and exclusion articles. After extracting 221 studies from English databases, 60 duplicates were excluded. After evaluating 161 papers for title and abstract, 77 were discarded. Following full-text analysis, 73 irrelevant publications were removed, while 11 research articles were chosen for the final study. All the studies were experimental design-based studies. The studies have used algal meals as an alternative to fish meal for the growth and development of commercial fishes such as Nile tilapia (Oreochromis niloticus) fish varieties from Saudi Arabia and Iran [16, 17], Gilthead seabream from Portugal [18], Juvenile tilapia from Florida [19], Atlantic Salmon (Salmo salar) from Australia [20], juvenile rainbow trout (Oncorhynchus mykiss) from Turkey [21], mullet (Mugil liza) from Brazil [22], Atlantic salmons from Norway [23], catla (Catla catla) and rohu (Labeo rohita) from India [24] and shrimp varieties such as Pacific white shrimp (Litopenaeus vannamei) [25].

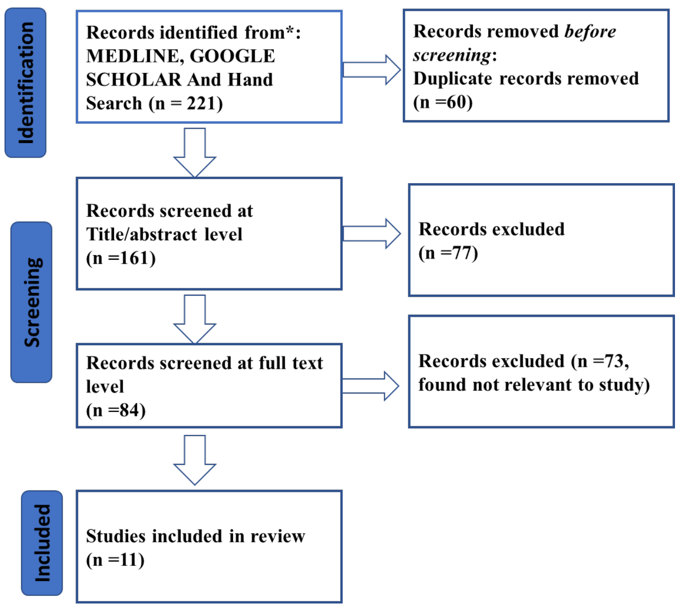

Fig. 1 PRISMA flow diagram for the systematic review which included searches of databases

3.2 Algae used in the preparation of algal feed

The following algae including Red algae (Gracilaria arcuate) collected from Red Sea beaches, Saudi Arabia [16], microalgae (Chlorella sp., DHA-rich Schizochytrium sp., Tetraselmis sp.) [18], Schizochytrium sp [19], Ulva ohnoi, diatom Entomoneis spp. [20], Ulva rigida collected from the shore of the Dardanelle waters, Turkey [21], Laurencia caspia from Caspian sea shore, Iran [17], Spirulina (A. platensis) [22], Nannochloropsis oceania [23], Spirulina platenis [24] and fermented algae, Schizochytrium sp [25].

3.3 Algal feed composition and impact on the growth of aquatic species

In a study conducted by Young et al., powdered red algae in various concentrations (20%, 40%, 60%) was used to replace fish meal. Maximum growth and gain in body weight of Nila tilapia was observed at a small concentration of 20% red algae. As the concentration of algae increased replacing the fish meal, the negative impact on the fish growth increased [16]. In a 12-week experimental study of three experimental diets (AD1, AD2, AD3), fish meal was replaced with 33% microalgae and had less amount of vegetable oils (11%). Gilthead seabream fish feed with AD1 and AD2 diets containing 0.4% microalgae and 20% less fish oil showed similar growth in comparison with the fish diets, thereby indicating that microalgal diet can replace fish meal diets partially [18]. Similarly, in an 8-week study, an algal meal diet containing 8.77% algae, 37% soya bean, 37% wheat, 10.9% bone and meat meal, 5% fish meal, 1.1% corn oil, and 0.2% vitamin and mineral premix demonstrated excellent growth in tilapia fillets. The diet improved the lipid profile of the fillets, indicating it can be used as an alternative to fish meal [19]. Norambuena et al. studied the impact of algal-derived products such as dried algal meal, Verdemin, and Rosamin on the growth of Atlantic salmon. The study found that feed containing 2.5% and 5% algal products neither had a positive nor negative impact on the feed efficacy and fish growth. However, in vivo analysis of Salmon fish bodies showed an increase in the concentration of omega-3 fatty acids [20]. Nevertheless, in another study on Atlantic Salmon fed on 20% defatted algae N. oceania meal showed a negative impact on the fish growth, feed intake and lipid profile of the fish. Only 10% of algal meal was found to have no major negative effects on fish [23]. In a study, rainbow trout fish were fed with a 12-week algal diet and later starved for 3 weeks. Fish fed with algal feed had only 50% body weight loss on starvation compared to other fish fed with fish meal. This suggested that algal diets can be useful during the rainy season or low market prices when fish are subjected to starvation [21]. In a 50-day study on Nile tilapia fish fed with alga L. caspica feed ranging from 0.5% to 2%, they demonstrated enhanced immunity and resistance to pathogens (S. agalactiae) [17]. Rosas et al. conducted a study on mullet fish. The fish were fed varying Spirulina meal concentrations and linseed oil (0 to 100%). The study findings show that 50% of experimental feeds can partially replace fish meal and can enhance the antioxidative response, growth, and quality of the fish [22]. Also, a study conducted on Indian carps-Rohu and Catla showed that algal feeds had no impact on Catla fish whereas 25% of algal feed had a significant impact on the growth of Rohu fish [24]. Similarly, fish oil was replaced with algal meal ranging from 0 to 75% in the shrimp feed mixture. The findings show increased intestinal lipase and fatty acid composition in shrimp, indicating that algal meal can replace fish oil in the feed mixture [25].

3.4 ROB analysis

Syrcle ROB analysis of all the selected studies showed no risk of bias in many domains. However, none of the studies conducted any sort of molecular analysis of the algal cultures used or followed a blinded strategy in their experiments to increase the validity of their experiments and decrease bias.

4. Discussion

To evaluate the possibility of replacing fishmeal entirely or in a portion of the diet of a significant aquaculture species, previously published literature was methodically chosen and examined using predefined criteria. The study's main objective was to offer detailed information about the effectiveness of several substitute algal protein sources. As a result, this research took into account a wide range of algae species and their effects on fish that are obtained worldwide.

Single-cell microalgae have several unique nutritional benefits above traditional fishmeal substitutes. Several microalgae species, including Spirulina, Nannochloropsis, and Chlorella, have protein levels ranging from 50% to 70%. Also, microalgae can synthesize all amino acids, and the functional proteins produced are equivalent to other alternative proteins, making them a well-balanced meal for aquatic animals. In addition, algae contain bioactive compounds such as polyunsaturated fatty acids, polysaccharides, vitamins, and minerals that can stimulate an immune response, enhance antioxidative response, resistance to pathogens and coloration in fishes and shrimps [26, 27]. Table 1 shows the list of studies included in the present analysis. Spirulina, Ulva, and Schizochytrium were the commonly used algal species in the present study for the preparation of algal meal in replacement of fish meal. Their ability to fully substitute fishmeal in the diets of some aquatic animals has been shown through growth performance studies, with positive impacts on the immune system, antioxidant response, and pigmentation of the fish species. Nevertheless, the algal feed substitution percentages in various trials varied, which can probably be due to variables including the type of aquatic animal and its stage of development, the amount of fishmeal added to the control diet, and the amount of microalgal protein.

Fish oil is another essential ingredient in aquafeed that provides functional micronutrients, particularly omega-3 fatty acids, which are critical for developing aquatic animals' immune systems, neurological systems, and specialized organs [28]. The high lipid content of microalgae makes them a viable alternative to fish oil in aquafeed. Oligomeric microalgae may attain a total lipid content of 45% of dry cell well weight (DCW) or, in the case of certain species, surpass 60% DCW when exposed to unfavorable conditions or under physiological stress [29]. In the present study also, Spirulina along with linseed oil [22], algal product Rosamin [20], and microalgae blend, i.e., Chlorella sp., DHA-rich Schizochytrium sp., Tetraselmis sp. [18] were found to enhance the fatty acid composition and poly-unsaturated fatty acids in different varieties of fishes. Schizochytrium species were found to enhance the fatty acid concentration in both fish and shrimps [19, 25].

The present study concludes that microalgae, which can be used for the production of biopolymers, can partially be used as an alternative to fish meal. However, only low concentrations of microalgae feeds were found to positively impact overall fish growth and body composition. The present study is limited due to sample size and considering different varieties of fish species with considerably different growth parameters.

5. Conclusion

Microalgae can be used not only for the production of biodegradable biopolymers/bioplastics, as well as an alternative feed to replace fish meal and fish oil. Only low dosages of algal feed were found to be effective. Further studies are required to comparatively analyze the efficiency of different types of microalgae in enhancing the growth and health of specific targeted fish species.

Data Availability Statement

No datasets were generated or analysed during the current study.

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Table 1 Summary of studies included in this analysis based on the inclusion

Aim	Alagae meal	Fish species	Meal preparation	Results	Conclusion	Reference
To determine the impact of red on the growth and body composition of Nila tilapia fish alternative to fish meal in a laboratory	Gracilaria arcuata meal	Nile tilapia, (O. niloticus)	Red algae were completely cleaned in freshwater and distilled water, allowed to sun dry for 48 hours, and then finely ground in a lab blender to a dried powder and given in three dosages-G20%, G40% and G60%.	Algae given as feed to fish showed a negative impact on growth parameters such as the final weight of fish, % of weight gain, and daily and specific growth rate. Furthermore, as the amount of algae in the diet increased, Gracilaria meals had detrimental effects on Nile tilapia development performance as well. Fish on diet G20 showed noticeably better growth performance than fish on either of the G40 or G60 diets. However, there were no appreciable variations in the feed utilization metrics feed conversion ratio) and protein efficiency ratio between the fish in the diet G20 group and the control group	Less than 20% of the red algae could be fed to Nile tilapia in place of fish meal. However, more research is required to determine the ideal amount of Gracilaria meal for Nile tilapia diets in order to enhance growth performance.	[16]
The objective of this study was to assess the effects on gilthead seabream development, nutrient utilization, tissue composition, and gene expression of biofortified diets, including microalgae, macroalgae, and selenium-rich yeast.	Microalgae-Chlorella sp., Tetraselmis sp. and DHA-rich Schizochytrium sp., Macroalgae-L. digitata and selenised yeast	Gilthead seabream	AD1 AD2 AD3 Fishmeal 10.0 10.0 10.0 Microalgae meal 4.6 4.6 4.6 Fish oil 4.4 5.5 5.5 Soybean oil 2.5 2.2 2.12 Rapeseed oil 5.0 4.3 4.3 Linseed oil 0.8 0.7 0.7 The remaining ingredients wheat, corn meal, vitamin premix are same in all the meals	Growth was comparable in fed mix of AD1, AD2, and control in 12 week study, suggesting that microalgae meal can partially substitute both fish meal and fish oil in seabream diets. However, AD3 inhibited fish growth, indicating that the supplementation of selenium yeast and L. digitata should be limited to less than 0.04% and 0.8 percent, respectively. Fish administered AD3 had lower lipid intake and decreased PUFA bioavailability, but their livers' elovl5 was upregulated, leading to a notable rise in muscle EPA + DHA as compared to fish fed CTRL. In fact, fish fillets fed AD2 and AD3 had the greatest EPA + DHA concentrations (0.7 g 100 g−1), which is significantly higher than the minimal amounts advised for human consumption. Fish fed the AD diets showed increased selenium retention and growth.	The investigated microalgae are rich in lipids and protein, with their polyunsaturated fatty acids are efficiently stored in the muscle of seabreams. Fish can benefit from fortification with selenium-enriched yeast, but more work has to be done to to develop fresh approaches for iodine fortification of fish.	[18]
To assess whether diets fortified with fish oil and algal meal (AM) can lower the n-6:n-3 ratio and enhance LC-PUFAs in market-size fish fillets and offal	Schizochytrium sp	Juvenile tilapia	Diets containing various levels of fish oils (1%, 3%, 5%) and algal meal (1.75%, 5.26%, 8.77%)	Fatty acid composition in the tilapia fish tissues increased with increasing concentrations of fish oil and algal meal. No significant difference in different production parameters between the fish oil meals and algal meals.	Overall, the study's experimental diets appear to be an effective strategy for increasing the healthy omega-3 content of tilapia fillets and yield value-added by-products.	[19]

Aim	Alagae meal	Fish species	Meal preparation	Results	Conclusion	Reference
To evaluate the potentials of algal products-Verdemin and Rosamin on growth and fatty acid profile of Atlantic Salmon two commercially available algae derived products (dry algae meal), Verdemin (derived from Ulva ohnoi) and Rosamin (derived from diatom Entomoneis spp.) for their possible inclusion into diet of Atlantic Salmon (Salmo salar).	two algal products Verdemin (derived from Ulva ohnoi) and Rosamin (derived from diatom Entomoneis spp	Atlantic Salmon (Salmo salar)	Algal products 2.5% and 5% singly or in combination fortified with a low percentage of fish meal and fish oil	The findings show that the addition of the algal products Rosamin and Verdemin at dosages of 2.5 and 5.0% had no discernible effects on the growth or feed efficacy of Atlantic salmon. Fish fed 5% Rosamin showed a rise in the amount of omega-3 long-chain polyunsaturated fatty acids throughout their bodies.	According to this study, verdemin and rosamin appear to have little promise for use in salmon feed when it comes to overall performance. Nonetheless, certain intriguing biologic actions were noted, which may be useful for particular applications and call for more carefully planned research.	[20]
To investigate the effects of red macroalgae extract (Laurencia caspica) on hematological, immunological, antioxidant, biochemical, and disease resistance against S. agalactiae in Nile tilapia for 50 days	L. caspica	Oreochromis niloticus	Algal meal 00.5%, 1%, 2%	In a 50-day study, blood parameters (WBC, hemoglobin, neutrophils), immunological variables ([IgM, C3), liver enzymes (ALP, ALT), and antioxidative enzymes (peroxidase, superoxide dismutase, catalase) were enhanced in Nila tilapia fishes. However, no significant difference between the control and experimental diet was observed at a 25-day interval. Also, increased resistance to pathogen S. agalactiae in fish fed with algal fed.	Feeding Nile tilapia with L. caspica algae extract improved the fish's blood, safety, antioxidant levels, and biochemical markers. Because it has a positive effect on S. agalactiae bacterial infection, the administration of this algae can be seen as a straightforward and promising approach to managing bacterial disease in aquaculture.	[17]
To assess supplementing dietary fish oil with Schizochytrium meal, either alone or in conjunction with Soybean oil and linseed oil, affects the intestinal lipase activity, growth, fatty acid composition, and antioxidant levels in Pacific white prawns, L. Vannamei	fermented algae, Schizochytrium sp	Pacific white shrimp (Litopenaeus vannamei)	diet 1- fish oil diets 2 & 3- algae-meal and soybean oil diet 4- algae meal, SBO and linseed oil (LSO) diet 5- microalgae meal and equal amounts of LSO and SBO diets 6 and 7-equal amounts of algae meal but with LSO or SBO diet 8-algae-meaL	L. vannamei growth and feeding efficiency did not differ noticeably across diet regimens. Muscle's fatty acid composition usually mirrored dietary fat content. Shrimp fed with diet meal 3 and 7 had much more muscle sub-epidermal adipose tissue, whereas shrimp on diets 7 and 8 had significantly more intestinal lipase. The dietary treatments had no effect on muscle lipid peroxidation. However, prawns fed diet 7 exhibited noticeably stronger antioxidant activity than those fed diet 1.	In conclusion, Algal meal can be an alternative to fish oil completely without major on the growth of shrimp.	[25]

Aim	Alagae meal	Fish species	Meal preparation	Results	Conclusion	Reference
To ascertain how nutritional algal supplementation affects rainbow trout, Oncorhynchus mykiss, during starvation in terms of weight loss and proximate composition.	Ulva rigida, Spirulina	Juvenile rainbow trout O. mykiss	Ulva rigida-5%, 10% Spirulina- 5%, 100% control	When the fish were starved for two or three weeks, their cumulative weight loss was over 50% less than that of the control group ($p < 0.05$). A comparison of the fish fed various algal diets throughout the starving period revealed no discernible changes in weight loss. For the duration of the starving period, the total protein and ash concentrations of each group remained constant. Changes in the liver fatty acid profiles were also noted, and fish in all treatments lost a considerable amount of total lipids.	Findings show that a small quantity of algae added to aquafeeds could be cost-effective in preventing fish from losing weight during brief fasts, which are occasionally required following a period of intense rain or when fish stocks are restricted.	[21]
To assess the impact of simultaneously substituting linseed oil and Spirulina (Arthrospira platensis) for fish meal and fish oil on growth of mullets	Spirulina (Arthrospira platensis)	mullets (Mugil liza)	Algal Spirulina meal and linseed oil (0%, 30%, 50%, 70%, 100%).	The colorimetric measurements revealed that the fillet coloration has improved ($p < 0.05$). Additionally, the study discovered that muscle had a 30% substitution for carotenoids. As the substitution level rose and the amount of these FAs in the diet decreased, the amount of highly unsaturated fatty acids in muscle decreased. When substitution with linseed oil levels was above 50%, elongation from 18:3n-3 to 20:3n-3 was observed. However, no desaturation to 20:4 or 20:5n-3 was found, which may suggest a specific n-3 HUFA need under the experimental conditions. Furthermore, we observed that the antioxidant capacity increased up to the SS70 treatment; the antioxidant capacity decreased with the SS100 treatment.	The study findings show that 50% of experimental feds can partially replace fish meal and can enhance the antioxidative response, growth and quality of the fish	[22]
This research aims to examine the growth, feed intake, gain, and health characteristics of Atlantic salmon that are given defatted Nannochloropsis oceania as a fishmeal substitute for 84 days.	Nannochloropsis oceania	Atlantic salmon	Algal meal-10%, 20%	The whole body and fillet proximate composition, as well as the hepatosomatic and viscerosomatic indicators, remained unaffected by the dietary interventions. The fish that were fed 20% algal meal also showed substantial differences in their digestibility as well as in their retention of lipid and energy. When compared to the control fish, the fish fed 10% algae had serum superoxide dismutase activity that was noticeably higher. Despite the lack of distal intestine inflammation caused by algae eating, changes in intestinal proteins following a 20% algal meal may indicate systemic physiological disruptions.	Feeds containing 20% alga had a negative impact on overall fish growth, metabolism, and health, whereas 10% algal feed does not have a major effect on fish and can be utilized as alternative feed.	[23]

Aim	Alagae meal	Fish species	Meal preparation	Results	Conclusion	Reference
To study the impact of of Spirulina platensis meal on the growth and body composition of two Indian major carps, Catla and rohu	catla, Catla catla and rohu, Labeo rohita	Spirulina platensis	Algal meal-25%, 50%, 75%, 100%	There was no significant change in catla's final weight at any dosage of Spirulina incorporation when compared to control diet. Nonetheless, rohu grew noticeably better when more than 25% of the fish diet was replaced with spirulina. Higher levels of Spirulina inclusion were associated with improvements in the specific growth rate and protein efficiency ratio reported in rohu, but in catla there was no significant difference between the treatment and control groups. The study revealed that a slight improvement in the digestibility in both species as the dosage of Spirulina incorporation increased. Protein and fat deposition were inversely correlated in the carcass composition. Fish-fed diets with spirulina generally had a much higher fat content.	The study showed how beneficial spirulina is for replacing fish meal in the diets of rohu and catla, either entirely or partly.	[24]

Supplementary Table 1 Risk of bias analysis of the selected studies

Sequence generation	Baseline characteristics	Allocation concealment	Random housing	Blinding	Random outcome assessment	Blinding	Incomplete outcome data	Selective outcome reporting	Other sources of bias	
Was the allocation sequence adequately generated and applied?	Were the groups similar at baseline or were they adjusted for confounders in the analysis?	Was the allocation adequately concealed?	Were the animals randomly housed during the experiment?	Were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment?	Were animals selected at random for outcome assessment?	Was the outcome assessor blinded?	Were incomplete outcome data adequately addressed?	Are reports of the study free of selective outcome reporting?	Was the study apparently free of other problems that could result in high risk of bias?	Reference
no	yes	no	yes	unclear	yes	unclear	yes	yes	yes	[16]
no	yes	no	yes	no	yes	yes	yes	yes	yes	[18]
no	yes	no	yes	no	yes	unclear	yes	yes	yes	[19]
no	yes	no	yes	no	yes	unclear	yes	yes	yes	[20]
no	yes	no	yes	no	yes	unclear	yes	yes	yes	[17]
no	yes	no	yes	no	yes	unclear	yes	yes	yes	[25]
no	yes	no	yes	no	yes	unclear	yes	yes	yes	[21]
no	yes	no	yes	no	yes	unclear	yes	yes	yes	[22]
no	yes	no	yes	no	yes	unclear	yes	yes	yes	[23]
no	yes	no	yes	no	yes	unclear	yes	yes	yes	[24]

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