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Effects of Dietary Microalgae Inclusion on Feed Utilization, Morphological and Chemical Characteristics and Body Health in Fish: Advantages and Disadvantages

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ABSTRACT

Dietary microalgae inclusion in small or large amounts to fish species is considered one of the sources for improving nutritional values and productive performances throughout the world. The microalgae are classified through light and electron microscopes into four groups: blue-green, green, golden and diatoms algae. The microalgae can be cultured in the laboratory under controlled conditions. Hence, the grown microalgae biochemical analysis of pigments, proteins and carbohydrates were differed over controlled conditions. Moreover, molecular biology concerning genes and genomes enabled to study ecology, evolution and physiology of microalgae. The fascinating field of microalgae genetic engineering was applied to produce invaluable microalgae compounds as omega-3 fatty acids. The microalgae and their purified compounds were used as direct nutritional sources or as indirect contributors through improvement of water quality and reduction of stress. The chemical composition of microalgae differed depending on nutrient availability for production and microalgae species. The microalgae invaluable compounds include “proteins, lipids, polysaccharides, polyunsaturated fatty acids, pigments, vitamins and other bioactive constituents”. The dietary microalgae and their purified compounds have been shown to provide beneficial effects in growth performance and body health. The roles of microalgae and their constituents as antioxidant, antibacterial, antiviral and anti-tumour materials have been well-established versus challenging problems. In this context, this review is designed to compile and discuss the advantages and disadvantages effects of microalgae on feed utilization, reproductive and therapeutic performances in fish.

Key words: Blood, Flesh color, Growth, Microalgae, Reproduction.

Microalgae due to their high nutritional values can be used as part of the solution for the global food crisis because the world population is expected to reach 9.7 billion in 2050 (Dorling 2021). Some microalgae species as *Chlorella, Dunaliella, Haematococcus, Schizochytrium* and *Spirulina* among thousands species are recognized as safe for human consumption in addition to their potential use for animal and fish nutrition as well (Almadani 2017; Senosy *et al*., 2017; Torres-Tiji *et al*., 2020). Microalgae are microscopic unicellular organisms, which can be found in both freshwater and seawater. Microalgae cultivation has a lower carbon footprint because it requires fewer resources including water, land, fertilizers in addition to consuming of microalgae the carbon dioxide during photosynthesis, which leads to reduction of greenhouse gas release to the atmosphere (Rahman *et al.,* 2023).

Dietary microalgae inclusion in small or large amounts to mammalian and fish species are considered one of the most important feed supplements for improving nutritional values and productive performances throughout the world (Abdelwahab *et al*., 2020; 2023). They gained prominence and limelight continuous interest because of their crucial roles for mammalian and fish species (Mohammed 2018, 2022; Ali *et al*., 2021; Al-Mafurji *et al*., 2022; Molina-Roque *et al.,* 2022). The roles of microalgae as direct nutritional sources might include supplying essential nutrients, improved growth and survival and enhanced fish quality (Wang *et al*., 2022a,b; Silva *et al*., 2023). Additionally, the roles of microalgae as an indirect contributor to performance might include improvement of water quality, live feed for larvae and reduction of stress (Amira *et al.,* 2021; Ballesteros-Redondo *et al*., 2023) (Fig 1).

Aquaculture is diverse and dynamic and fastest-growing field and is considered one of the most important system worldwide for protein production supplied to human diets (Ekasari *et al*., 2016, 2023). The demands for microalgae and their purified components have increased steadily due

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to their sustainable sources and functional properties. Protein sources and levels are the main components of diets for aquatic organisms (Abdelwahab *et al.,* 2023; Randazzo *et al.*, 2023). Fish feed is represented more than half of the costs of the fish project due to the high price of fishmeal as source of protein for aquatic organisms. Therefore, replacing fishmeal with different microalgae species as protein sources is required to face the increasing demand for fish feeding (Almadani, 2017). The microalgae culture and inclusion represents advantages and disadvantages to environment and aquaculture system (Garcia-Vaquero and Hayes 2016; Wilfart *et al.,* 2023). Hence, review article is designed to compile and discuss the effects of microalgae on feed utilization, body weight gain, reproductive and therapeutic performances in fish species.

The current review was designed according to the procedure approved by Deanship of Scientific Research, King Faisal University, Saudi Arabi from October to February 2024. The article present the information of microalgae concerning direct nutritional resources, indirect contributor to fish performance, feed utilization and growth performance, reproductive performance, blood profile and plasma metabolites, immunity and therapeutic performances in addition to disadvantages of microalgae inclusion for aquaculture system. The materials were collected from PubMed, science direct and google scholar research engines.

The results of microalgae and purified compound on productive and reproductive performances and body health were discussed in this article. The macromolecules and micronutrients of microalgae are played crucial roles for improvement of feed utilization and body weight gain, reproductive performance and immunity and body health (Dineshbabu *et al*., 2019; Altmann *et al*., 2020; Wang *et al*., 2022a; Sánchez *et al*., 2023; Randazzo *et al*., 2023). Feeding diets high in microalgae can result in higher feed utilization, increased body weight and better survival rates compared to traditional fishmeal-based diets (Amira *et al.,* 2021). Besides, certain dietary microalgae can positively influence the meat quality of fish. They can boost the levels of desirable fatty acids, like ω -3 fatty acids, leading to tastier and healthier fish for consumers (Karageorgou *et al*., 2023). On the other hand, challenging problems of supplementing microalgae for aquaculture systems were reported in several studies (Lu *et al.,* 2023).

Effects of microalgae as direct nutritional resources to fish species

Prominence and heightened continuous interest for the use of microalgae as a novel feed supplement for nutritional purposes in different species of animals and fish has been

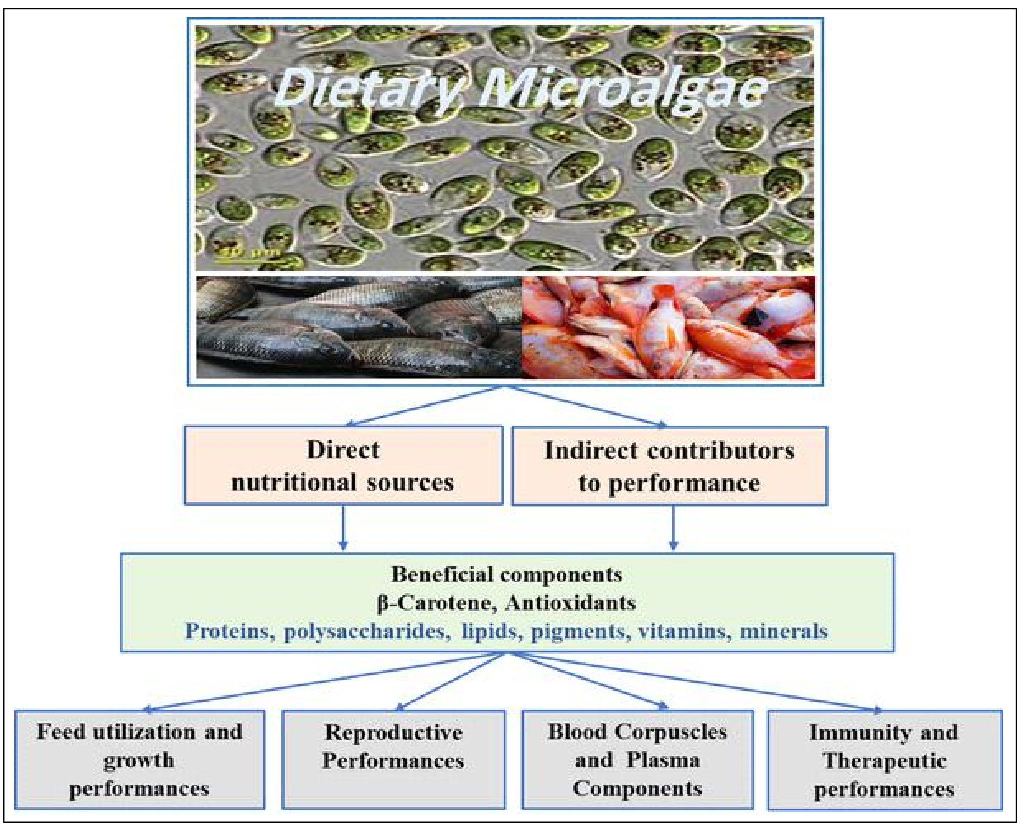

Fig 1: Effects of dietary microalgae and their constituents as direct nutritional sources and indirect contributors to performance of fish.

indicated because of their unique chemical composition (Almadani 2017; Senosy *et al.*, 2017; Nagarajan *et al.*, 2021). Microalgae contain protein, fat, fibers, vitamins, minerals, â-carotene and antioxidant compounds (Mohammed 2018; Alagawany *et al.*, 2021; Nagappan *et al.,* 2021). The chemical composition of microalgae varies depending on nutrient availability for production and species of microalgae (Tham *et al.*, 2023). It is a fascinating blend of macromolecules and micronutrients. *Dunaliella salina* as example, a single-celled green microalga, contains more than 8.4% moisture, 54.1% crude protein, 0.8% fiber, 11.4% total lipid, 18.4% ether extract and 6.6% ash (Almadani 2017; Mohammed 2018). Microalgae contain, in some cases, a higher protein amount than soybean, corn and wheat (Kratzer and Murkovic 2021). Additionally, microalgae contain carotenoids, phenolic compounds, pigments, essential vitamins and minerals, which make the microalgae treasure troves of bioactive compounds with a diverse range of potential health benefits and applications (Cuellar-Bermudez *et al.*, 2015; Gong and Bassi, 2016; Al Mufarji *et al.*, 2022).

On the other hand, the unfavorable components of microalgae include cellulose and hemicellulose and anti-nutritional factors were reported in several studies (Alagawany *et al.,* 2021; Seghiri *et al.,* 2019; Metsoviti *et al.,* 2020). The complex polysaccharides leading to poor digestibility, excess heavy metals and presence of anti-nutritional factors like phlorotannins, lectins and phytic acids, amylase inhibitors and trypsin inhibitors of microalgae were indicated in several studies (Garcia-Vaquero and Hayes 2016; Wilfart *et al.,* 2023).

Effects of microalgae as indirect contributor to fish performance

Microalgae might be used as indirect contributors to performances through improvement of water quality, live feed for larvae and reduction of stress (Abdelwahab 2023; Ballesteros-Redondo *et al.*, 2023). Firstly, improving water quality can be achieved through microalgae, which work as natural bio-filters by removing excess nutrients and harmful bacteria (Amira *et al.,* 2021). Microalgae-based nutrient removal for aquaculture waste has been confirmed in several studies (Nie *et al.,* 2020) in addition to biodesalination of water using various microalgae species in various conditions (Esmaeili *et al.*, 2023). Progress on microalgae cultivation in wastewater for bioremediation and circular bioeconomy is indicated (Satya *et al.,* 2023). This results in a healthier environment for fish, rising their immune system and reducing disease susceptibility.

Secondly, many larvae of fish depend on tiny zooplankton as their primary feed sources. Some microalgae species can serve as excellent substitutes for traditional live feed, ensuring proper nutrition and survival for young fish. Finfish larviculture requires a diet rich in fatty acid profiles including omega-3 fatty acids and docosahexaenoic acid (Ballesteros-Redondo *et al.*, 2023). The significance of microalgae to consume CO_2 has been reported (Olabi *et al.*, 2022). Microalgae are efficiently capable of fixing CO_2 and simultaneously producing biomass for multiple applications, which is considered one of the most promising pathways for carbon capture and utilization (Xu *et al.,* 2023). In addition, Kumaran *et al.,* (2023) indicated agriculture of *Chlorella vulgaris* for producing polyunsaturated fatty acids through palm oil mill effluents. Lastly, certain microalgae possess potential anti-stress properties, which can help to alleviate stress in farmed fish, promoting better growth and performance (Al Mufarji *et al.*, 2022; Mishra and Tiwari 2022; Fan *et al.,* 2022).

Effects of microalgae on feed utilization and growth performance

There is a heightened continuous interest for the use of microalgae as a dietary supplement for different animal and fish species (Senosy *et al.*, 2017; Nagappan *et al.,* 2021; Abdelwahab *et al.*, 2023; Orzuna-Orzuna *et al.*, 2023). In general, certain microalgae species harbor prebiotic fibers that nourish gut bacteria, improving digestion and nutrient absorption (Wang *et al.*, 2022a; Silva *et al.*, 2023). This allows fish to extract more energy and nutrients from their feed, leading to better growth performance and flesh quality (Molina-Roque *et al.*, 2022). Besides, microalgae can add exciting flavor and aroma to fish feed, increasing feed intake and promoting optimal growth depending on microalgae species, biotechnological treatment of microalgae and level of supplementation (Molina-Roque *et al.*, 2022; Khoo *et al.*, 2023; Samuelsen *et al.*, 2024).

The studies were differed in their experimental design conditions concerning the duration of study, microalgae species and the level of supplements (Mohammed 2018; Al Mafurji *et al.,* 2022; Al Mafurji *et al.*, 2022; Abdelwahab *et al.*, 2023). Carneiro *et al.*, (2020) found that replacement of fishmeal by Chlorella meal resulted in an increase in the final weight of zebrafish. In our lab, dietary *D. salina* were fed to Red Tilapia for four months as replacement of fishmeal at level 33.0, 66.0 and 100.0%. Replacement of fishmeal with *D. salina* at 33.0% level gave similar results as control diet concerning feed efficiency, body weight gain, morphological and chemical profiles versus 66.0 and 100.0% levels, which were mostly decreased the aforementioned parameters. The negative effect of level 66.0 and 100.0% *D. salina* could be owing to problems of palatability (Walker and Berlinsky 2011) in addition to imbalances in diets. Fish flesh color is the first character to be evaluated by consumer and relevant to market acceptance (Ünal Şengör *et al.*, 2019). In our study, *D. salina* supplementation resulted in significant improvement of flesh color upon 66.0 and 100.0% dietary inclusion versus control and 33.0% *D. salina* diets. This could be owing to pigment contents including chlorophylls, carotenoids, phycobiliproteins, xanthophylls (Roy and Ruma 2014), which constitute 3-5% of the dry algae biomass (Venkataraman and Becker1985). Collectively, microalgae show a promising potential to replace fishmeal and fish oil that was used mainly in the aquaculture feed. This is because both freshwater and marine microalgae contain a high composition of protein, lipid, carbohydrates and other bioactive compounds, which are essential for fish growth, development and resulting flesh quality.

Effects of microalgae on reproductive performance

Microalgae as mentioned earlier are contained high protein, fats, essential vitamins and minerals, which are crucial for maintaining healthy reproductive organs and functions. The positive effect of microalgae on reproductive performance has been confirmed in several earlier studies in fish and mammalian species (Posser *et al.*, 2018). Carneiro *et al.* (2020) found that replacement of fishmeal by *Chlorella meal* resulted in improvement in the reproductive performance of zebrafish. The inclusion of *D. salina* of our studies (Senosy *et al.*, 2017; Mohammed 2018) in diets fed to Boer goats and mice indicated significant increase of FSH, LH, estrogen and progesterone hormone values. In addition, acceleration of ovarian follicle development and increased ovarian follicle numbers were obtained as well. The aspirated oocytes from ovarian follicles were highly cumulus-enclosed but they were not differed in maturation processes including germinal vesicle breakdown (GVBD), metaphase I and metaphase II stages as well as the maturation rates. Beyond the maturation, the fertilized oocytes were highly developed to the blastocyst stage compared to control diet. In conclusion, microalgae can be powerful products in enhancing fish reproductive performance through providing essential nutrients, reducing stress and improving eggs and spermatozoa quality. However, the current challenges and future perspectives are to avoid their potential negative effects through toxins, which necessitates careful selection and management to promote reproductive and sustainable fish farming.

Effects of microalgae on mlood profile and plasma metabolites

Several studies were explored the effects of different microalgae species fed to different fish and mammalian species on blood profile and plasma metabolites (Almadani 2017; Senosy *et al.*, 2017; Al Ali *et al.*, 2021; Mafurji *et al.*, 2022). Carneiro *et al.*, (2020) found that replacement of fishmeal by *Chlorella* meal resulted in an improvement in total protein, total cholesterol, low-density lipoprotein and triglyceride values. The conclusion of *D. salina* in our study up to 5.0% (Senosy *et al.*, 2017; Mohammed 2018; Ali *et al.*, 2021; Al Mafurji *et al.*, 2022) in diet fed to Boer goats and mice indicated significant improvement of blood (RBCs, WBCs, Ht and Hb) and plasma profiles (total protein, albumin, glucose, total cholesterol, BUN, AST, ALT) over feeding. Such positive effects of microalgae on blood and plasma metabolites is owing to several factors as the high protein content in microalgae, which enhance amino acid profiles in blood and promote protein synthesis. In addition, certain microalgae species are rich in omega-3 fatty acids resulting in improvement of cholesterol values. Besides, the high content of antioxidant compounds in microalgae can scavenge free radicals and oxidative stress resulting in an increase of antioxidant enzymes in blood and protecting cells from damage. Lastly, the microalgae can be good sources of essential minerals like iron, calcium and magnesium, leading to optimal mineral levels in plasma and maintain physiological functions.

On the other hand, blood and plasma values including RBCs, hematocrit, total protein and glucose were not differed of 33.0, 66.0 and 100.0% *D. salina* inclusion instead of fishmeal indicating negative effects with increasing the levels of *D. salina* microalgae. These negative effects could be attributed to imbalances in the diet upon higher inclusion of *D. salina*.

Effects of microalgae on immunity and therapeutic performances

Diseases are the most critical factors restricting the sustainable development of aquaculture and microalgae can strengthen the immune protection of aquatic organisms. Microalgae species can offer an alternative that could strengthen the immune status due to their nutritional and bioactive compounds. Sánchez *et al.*, (2023) found that *N. gaditana* and *Schizochytrium* spp microalgae inclusion can stimulate innate humoral antibacterial components, increase phagocytic cells and immature erythrocytes in blood of *S. salar*. Additionally, Randazzo *et al.* (2023) explored the effect of a microalgae blend (*Tetraselmis suecica* and *Tisochrysis lutea*) as supplements to replace 10% protein of a fish meal-free on physiological status and gut health. The microalgae blend led to a modulation of inflammatory gene expression in the distal intestine.

Certain microalgae species are rich in omega-3 fatty acids resulting in improvement of cholesterol values and reduce inflammation in plasma. Microalgae can stimulate the immune system, resulting in increased levels of immune cells and antibodies in blood and improving disease resistance (Fan *et al.*, 2022). Microalgae are brimming with antioxidants that combat free radicals and oxidative stress, strengthening the fish's immune system to fight disease and prevent cell damage (Gatlin and Yamamoto 2022). Certain microalgae harbor prebiotic fibers that nourish gut bacteria, enhancing digestion and promoting the production of immune-boosting compounds. Certain microalgae contain bioactive compounds like phycobiliproteins and beta-glucans that directly activate immune cells and enhance their responsiveness to infections.

Challenging problems of microalgae inclusion for aquaculture system

The challenging problems of applying microalgae for sustainable aquaculture system include assimilation of heavy metals and nutrients, unfavorable components of

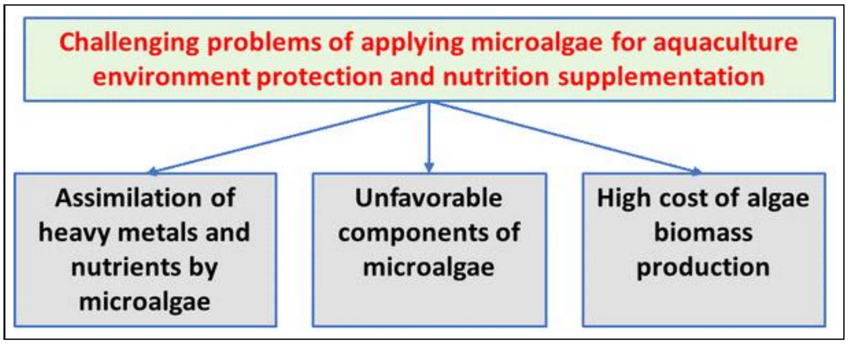

Fig 2: Challenging problems of applying microalgae for aquaculture system (Adapted from Lu *et al.*, 2023).

microalgae in addition to the high cost of algae biomass production (Fig 2) (Han *et al.,* 2019; Lu *et al.,* 2023).

Firstly, microalgae have good capacity of adsorbing heavy metals in water because of the negative charge on the surface of algal cells (Pugazhendhi *et al.,* 2019; Leong and Chang, 2020; Alagawany *et al*., 2021). Therefore, consuming fish microalgae adsorbed heavy metals will result in accumulation of heavy metals in blood and meat of fish. Furthermore, death and decomposition of microalgae will return the absorbed or adsorbed heavy metal to aquaculture water, which make them pollutant sources. In addition, continuous harvesting of microalgae from the water of aquaculture is time-consuming and very expensive (Li *et al*., 2021). Secondly, unfavorable components of microalgae as cell wall fiber (fiber 4.07-9.43%) and anti-nutritional factors were reported in several studies (Seghiri *et al*., 2019; Metsoviti *et al*., 2020), which reduces the nutrient digestibility and growth rate of fish (Ansari *et al*., 2021) and threat farm fish as well (Cannell *et al*., 1988; Ishihara *et al*., 2006). Thirdly, the high production cost of microalgae biomass compared to the prices of fishmeal is confirmed (Guccione *et al*., 2014; Karan *et al*., 2022). Therefore, the commercialization of microalgae as feed and food commodity for fish and mammalian species is not mature yet (Guccione *et al.,* 2014). Lastly, the problems of microalgae palatability were also mentioned in several studies (Walker and Berlinsky 2011). Hence, the emerging technologies of employing algae and microorganisms to promote feed utilization, growth and reproductive performance and body health of fish is prospective future studies.

CONCLUSION

While dietary microalgae showed immense potential for improving growth and reproductive performances, blood metabolites and body health of fish, some challenges require further research including the high production costs, the optimal microalgae species and dietary levels for different fish species and the microalgae free of harmful contaminants. Collectively, dietary microalgae inclusion in aquaculture is fastest-growing fields offered a promising avenue for promoting sustainable, healthy and high-performing fish production.

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Conflicts of interest

There is no conflict of interest for authors to declare.

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