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Review Article

Super Food Spirulina and its Growing Potential in Aquaculture

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ABSTRACT:
The sustainability of aquaculture depends on the continuous and improved nutrients of feed. The rising prices and lack of effectiveness of fish meal and fish oil are pushing feed manufacturing industries to find out alternative diet supplements which are not only more nutritious for fish but also of plant origin with a cost-effective range. So, blue-green filamentous microalgae Spirulina is one of the most promising sources of protein and the best alternative to expensive animal proteins in fish feed. Spirulina possesses excellent nutritional supplements for almost all kinds of fish species. As its nutritional component improve growth, carcass composition, immunological responses, disease resistance, reproductive function, and pigmentation. Spirulina can also utilize nutrients efficiently and eliminate heavy metals from aquaculture discharge. This water purification procedure not only lowers the cost of raw materials for growing Spirulina, which can be utilized as a dietary supplement in the aquaculture, but it also improves water quality and reduces water usage in high-density fish farms with restricted water exchange. Integrating Spirulina into traditional Recirculating Aquaculture Systems (RAS) appears to be a great fish farming integrated technique.

Keywords: Spirulina, Dietary Supplements, Protein profile, Recirculating Aquaculture Systems

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INTRODUCTION

The blue-green microalgae Spirulina is considered the most primeval form among microbes. The Aztec civilization used it for the first time as an endurance booster and now-a-day spirulina is devoured as a staple diet itself. The high concentration of nutritional components in it like vitamins, proteins, fatty acids, minerals, and the absence of cellulosic wall make it ingestible and tastier. This nutritious diet supplement is capturing international acceptance and growing commercial production demands for fish feeds. In aquaculture, Spirulina is one of the best alternative sources of proteins. Because aquaculture's popularity as a primary source of animal protein is growing by the day so, this is the need of the hour to promote fish farming to compensate for fish production demands (FAO, 2018).

Spirulina is a nutraceutical dietary supplement, but its potential health benefits have piqued interest in recent years as a potential source of medicinal chemicals (Sushma et al., 2021). In vitro and in vivo research have shown that spirulina has therapeutic qualities such as anti-inflammatory, immunomodulatory, and anti-oxidative (Hirahashi et al., 2002; Reddy et al., 2003; Hirahashi et al., 2002). Spirulina feed additives use in poultry; hatcheries, aquariums, and aquaculture are being promoted by several countries on a daily basis. Another key concern in fish aquaculture is water quality (Prema, 2009). Spirulina has recently gained popularity as a possible option for cleaning wastewater, particularly effluent from fish farming operations (Kamilya et al., 2006). Spirulina appears to be an ideal integrated strategy for fish aquaculture, as it may be used as a dietary supplement as well as to treat effluent for water quality control (Zhang et al., 2020). So, owing to the Spirulina feed use, cultivation methods at a large scale with different species are always an area of interest.

NUTRACEUTICAL CHARACTERISTICS OF SPIRULINA

Chopra & Bishnoi (2008) isolated Spirulina from freshwater samples, and later on, it is identified as a symbiotic, multicellular blue-green filamentous cyanobacterium (Ciferri, 1983). Spirulina is photosynthetic and phycocyanin is their principal photosynthetic pigment, however, they also contain chlorophyll a and carotenoids (Chen et al., 1996; Leema et al., 2010). Spirulina has more high-quality protein than other regularly used plant sources such as dry soybeans, peanuts, or cereals. It is a complete protein, having all essential amino acids. Polyunsaturated fatty acids (PUFAs) are abundant in *Spirulina*, accounting for 1.5–2.0 % of the total lipid (5–6%). Spirulina is particularly high in -linolenic acid, linoleic acid, stearidonic acid, eicosapentaenoic acid, docosahexaenoic acid, and arachidonic acid. Vitamins B1, B2, B3, B6, B9, B12, C, D, and E are all present in Spirulina. It contains potassium, calcium, chromium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium, and zinc, among other minerals as shown in table 2. Chlorophyll a, xanthophyll, beta-carotene, myxoxanthophyll, zeaxanthin, canthaxanthin, diatoxanthin, 3-hydroxyechinenone, beta-cryptoxanthin, oscillaxanthin, and the phycobiliproteins c-phycocyanin and allophycocyanin are among the colors found in Spirulina (Jung et al., 2019).

Table 1: General nutrient composition of Spirulina (Grosshagauer et al., 2020)

Composition	**Quantity (%)**
Proteins	55- 70%
Carbohydrates	15-25%
Lipids	6-8%
Minerals	7-13%
Humidity	3-7%
Dietary fibers	8-10%

Table 2: Amount of different components in Spirulina

Type of Vitamin	**Quantity**	**Minerals**	**Quantity**
Vitamin A	23000 IU	Calcium	70 mg
Vitamin B1	0.35 mg	Selenium	10 g
Beta-Carotene	14 mg	Germanium	60 g
Vitamin B2	0.40 mg	Copper	120 g
Vitamin C	0.8 mg	Manganese	0.5 mg
Vitamin B	3 1.4 mg	Iron	15 mg
Vitamin D	1200 IU	Chrome	25 g

Vitamin B6	60 g	Phosphorus	60 mg
Vitamin E	1.0 mg	Iodine	55 g
Folic acid	1.0 g	Magnesium	40 mg
Vitamin K	200 g	Sodium	90 mg
Vitamin B	12 20.0 g	Zinc	0.3 mg
Biotin	0.5 g	Potassium	140 mg
Pantothenic acid	10.0 g		
Inositol	6.4 mg		

Spirulina's biochemical composition varies depending on the growing conditions, particularly the salinity of the growing medium; it develops in freshwater (pH 7) as well as very alkaline settings (pH 9–11) in tropical and subtropical climates (Ciferri, 1983; Ayala & Bravo, 1982). According to Vonshak (1997) Salt-adapted cells showed a different biochemical composition, with less protein and chlorophyll and more glucose.

SPIRULINA AS A DIETARY SUPPLEMENT OR PROTEIN REPLACEMENT

For a fish diet, fishmeal is one of the most nutritionally complete ingredients and it's tough to replace it, especially for carnivorous fish that need a lot of protein in their diet (Tacon & Metian 2008; Oliva-Teles 2012). Gatlin et al. (2007) have examined proteic substances that could replace fishmeal in terms of quality without impacting cultured organism development and health. In comparison to other typical fishmeal alternatives such as soybean meal, Spirulina meal has a high nutritional value due to its high protein value, as well as its amino acid and fatty acid profile, as well as its various bioactive qualities, making it one of the best options to replace fishmeal as shown in table 3.

Table 3: Show Comparison of different feedstuff (National Research Council (2011)

Ingredients	Fishmeal	Soybean meal	Spirulina meal
Crude protein (%)	64.5	48.5	57.5
Crude lipids (%)	9.6	0.9	7.7
Ash (%)	19.0	5.8	5.8
Crude fibre (%)	0.7	3.4	3.7
NFE (%)	6.2	41.4	34.7
Lysine (%)	4.81	2.83	3.03
Methionine (%)	1.77	0.61	1.15
Histidine (%)	1.78	1.30	3.21
Arginine (%)	3.66	3.60	1.09
Threonine (%)	2.64	2.00	2.97
Leucine (%)	5.00	3.42	1.28
Isoleucine (%)	3.06	2.60	0.81
Cysteine (%)	0.61	0.71	0.29
Phenylalanine (%)	2.68	2.70	0.80
Tyrosine (%)	2.10	1.25	0.54
Tryptophan (%)	0.67	0.70	0.93
Valine (%)	3.26	2.70	3.51

The enhancement in the growth of fish occurs by Spirulina due to the presence of its prebiotic effect, high enzymatic activity, high vitamin content, antioxidant and immunostimulant activity (Ramakrishnan et al., 2008; Nandeesha et al., 2001; Adel et al., 2016; Belay et al., 1996; Ravi et al., 2010). Spirulina improves the growth of a variety of fish species, including tilapia and carp. Spirulina might replace up to 75% of the protein in the diets of juvenile *Nile tilapia,* according to Velasquez et al. (2016), with no negative impacts on growth or blood chemistry. Its high quantities in the diet produced similar effects on carp (Nandeesha et al., 2001). Therefore Spirulina is an excellent plant protein for replacing animal-derived proteins in the diets of tilapia and carp fish. Spirulina has been discovered as a key component in tilapia's natural diet (Velasquez et al., 2016). However, in excess amounts, Spirulina causes hindrance in the growth rates of silver sea bream and produces prooxidant activity in rabbits and chickens (El-Sayed 1994; Macari et al., 2020; Dal Bosco et al., 2014). The effect of Spirulina on fish development performance appears to be species-specific as the nutritional habits of the different organisms considerably affect the digestibility, retention, and absorption of nutrients differently (Santigosa et al., 2008).

EFFECT OF SPIRULINA ON FISH REPRODUCTION

Many freshwater ornamental fish fed non-standard brooder diets have low reproductive yields due to the effect of nutrition on reproductive performance (Izquierdo et al., 2001). Improved diets that include nutritious elements like Eicosapentaenoic and Arachidonic Acid can help fish reproduce better (Fernandez-Palacios et al., 1995). Linoleic and linolenic acids, which are precursors to Arachidonic Acid, are abundant in Spirulina. Arachidonic acid is required for the synthesis of prostaglandin, which is involved in steroidogenesis, oocyte maturation, and ovulation (Pati & Habibi, 2002).

Khanzadeh et al. (2016) reported that *Trichopodus trichopterus* fed meals enriched with *A. platensis* achieved the initial stage of spawning in less time than those fed simply fish meal. The total egg production of yellow tail cichlids (*Pseudotropheus acei*) was significantly improved by dietary supplementation of 2.5 percent Spirulina compared to those fed the cichlid's natural diet. Furthermore, Spirulina meal could be utilised to replace up to 10% of dietary fish meal without negatively impacting reproductive performance. So, reproductive performance is clearly improved by eating Spirulina. Essential fatty acids, ascorbic acid, and carotenoids have been shown to affect egg quality and production. In yellow tail fish, the addition of dietary carotenoids has been found to improve broodstock performance (Watanabe & VassalloAgius, 2003). Because Spirulina is high in the nutrients required for fish reproduction, this improvement can improve reproductivity.

SPIRULINA ACT AS AN IMMUNOSTIMULANT

High-density fish farming has become popular because of the increasing public demand for fish and the restricted availability of fresh water and land. Due to overcrowding and the related inadequate sanitation or nutrition in poor physiological settings, one of the most common difficulties connected with high-density fish farming is the spread of diseases like as bacterial infections and dropsy (Trenzado et al., 2008). Chemotherapy, vaccination, and feeding immunostimulants are all possible ways to make farmed fish less susceptible to infectious illnesses. However, there are only a few approved chemicals for chemotherapy, and they cause additional stress to the fish, and vaccines are only effective against a small number of diseases in a small number of fish species (Cain et al., 2003). Immunostimulants may defend against infections by boosting nonspecific defence mechanisms, which are crucial for stress and illness reduction (Talpur et al., 2013).

Sakai 1999 found that oral administration of certain chemicals like lipopolysaccharide, chitin, and glucan has been shown to boost fish immune defences. Spirulina has been shown to stimulate the immune systems of a variety of fish species, including channel catfish, African sharp tooth catfish, carp, rainbow trout, and big sturgeon (Duncan & Klesius 1996; Watanuki et al., 2006; Abdel-Tawwab & Ahmad 2009; Ragap et al., 2012; Yeganeh et al., 2015; Adel et al., 2016). *A. platensis* act as antimicrobial agent in fish feeds (Pradhan et al., 2014).

It is observed that there is significant change in immunological measures such as white blood cell count by using *A. platensis* for boosting the immune response in rainbow trout. The antibacterial activity of *A. platensis* was similarly elevated in the skin mucus of great sturgeon (Adel et al., 2016). Lysozyme in the blood, in addition to WBC and skin mucus, performs a significant bactericidal function in the non-specific defence against infections. In high-density fish farming, high lysozyme activity may be beneficial since it defends against large bacterial loads (Grinde et al., 1988). Finally, Spirulina's immunostimulatory effects have been discovered in a variety of fish species. Spirulina boosts phagocytic activity, lysozyme activity, bactericidal activity, and WBC levels in fish, stimulating their immune systems. It can control the expression of cytokine genes in tilapia leucocytes, which operate as signalling molecules in the immune system (Khalil et al. (2017).

SPIRULINA AND AQUACULTURE SYSTEM AS AMALGAMATED CO-CULTURE TECHNIQUE

The conventional cultivation methods of Spirulina generally include open pond culture, photo bioreactors, and mixed systems, (Soni et al., 2017). The Different aspects of Spirulina production have been studied in order to make it a more efficient economic. Recently, for high-density fish farming a reticulating aquaculture system is designed with low water exchange (Ebeling & Timmons 2012). Biological filters are used to eliminate the high nutrient concentrations induced by the accumulation of feed residue and fish excreta. On the other hand, these are only capable of converting ammonia and nitrite to nitrate, which is less harmful than ammonia.

Water replacement or denitrification systems can remove nitrate, but these systems are complicated and unsuitable for large-scale aquaculture (Otte & Rosenthal 1979). Because of the ability to remove inorganic nutrients effectively at a low cost, using Spirulina to remove nutrients appears to be a viable alternative. Furthermore, despite the fact that Spirulina is ineffective in removing nitrite, high nitrite levels can be prevented by combining Spirulina treatment with a RAS that can convert nitrite to nitrate by biological mechanisms ((Kamilya et al., 2006; Otte & Rosenthal 1979).

The water in a tank that has been set up for fish cultivation does not include excessive quantities of nutrients (nitrate and phosphate). To remove suspended contaminants, the water from the fish tank can be pumped into a mechanical filtration drum, and excess ammonia and nitrite can be removed further using bacteria in a biological filtration tank. After a series of treatments, including carbon dioxide removal, UV light disinfection, and oxygen injection, water is returned to the fish tank through first valve. When the nutrient levels in the effluent are high, the water discharged from the biological filtration tank can be pulled into a Spirulina tank through second valve for additional nutrient removal by Spirulina biological uptake. All nutrients are reduced to optimal levels by these procedures. Spirulina biomass is then collected via a 380–500 mesh sieve (as shown in fig.1) for use it as a fish feed supplement and the filtrate is disinfected and oxygenated before being reintroduced to the fish tank.

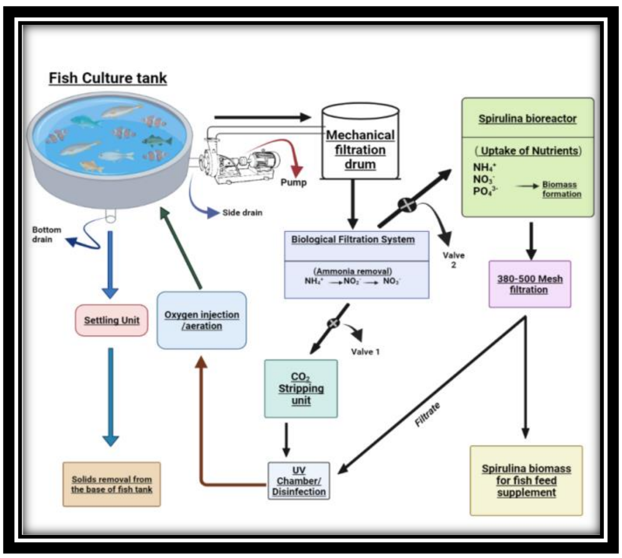

Figure 1: The proposed integrated co-culture techniques of Spirulina and fish

Madkour et al (2012) found that the use of urea in Spirulina production reduces the quantity of protein in Spirulina, which has an impact on its market value while Chang et al. (2013) used human urine as an ammonium source in the synthesis of Spirulina and found that by just adding carbon sources, it is possible to manufacture it without altering yield or nutritional quality.

The traditional medium (Zarrouk's) used to culture Spirulina was replaced with a culture medium containing fertilisers commonly used in agriculture, and it was discovered that the quality of the Spirulina was unaffected, lowering the cost of 1000 L of culture medium from $80 to $16, or 1/5 of its total value (Raoof et al., 2006). Finally, the utilization of enhanced Spirulina strains for the production of these microalgae under various environmental circumstances is a significant aspect that could lead to the use of Spirulina meal in aquaculture feeds being practical (Rajasekaran et al., 2015; Liu et al., 2016). To make this ingredient swap, enough Spirulina meal must be produced at a cost that is comparable with other feeds.

ROLE OF SPIRULINA IN EFFLUENT WATER MANAGEMENT

Using wastewater for Spirulina cultivation can be a sustainable alternative to present techniques, as long as the water remnants are not hazardous to animals. To boost the amount of Spirulina generated, Cheunbarn and Peerapornpisal (2010) employed diluted (10%) pig effluent. Cultivation of Spirulina in alternative media using industrial effluent from a confectionery industry, proving that growing Spirulina in alternative media can result in normal growth without compromising its nutritional characteristics (El-Kassas et al., 2015). Variation in the culture medium of microalgae can define its chemical composition and biomass output because of the utilization of alternative nutritional sources that can minimize the costs of Spirulina production (Jiang et al., 2015).

Removal of heavy metals- Heavy metal contamination of aquatic ecosystems is a major environmental concern because of their toxicity and tendency to bio-accumulate at multiple trophic levels (Cheng et al., 2017). Heavy metal contamination has been discovered in several economically important fish species. Cadmium, copper, and zinc accumulated in the muscular tissue of gilt-head bream (Dural et al., 2007). Heavy metals produce mutagenic and carcinogenic reactions, as well as morphological, physiological, and cytogenetic alterations in fishes. In *Channa punctatus*, Yadav and Trivedi (2009) discovered the genotoxic potential of three common heavy metals, mercury, arsenic, and copper, even at sub-lethal quantities.

Spirulina biomass is used as a biosorbent to remove heavy metals from aquatic environments. *A. platensis* to adsorbs cadmium and nickel ions (Celekli and Bozkurt 2011). *A. platensis* is capable of efficiently removing lead from industrial effluent, with a biosorption rate of up to 84.3% (Malakootian et al., 2016). Commercial Spirulina powder was used to demonstrate the feasibility of recovering cerium from industrial wastes; the greatest biosorption absorption was 38.2 mg g (Sadovsky et al., 2016). This could be an environmentally beneficial solution for bioremediation of the natural environment. Chromium is also removed from industrial effluent using a water-soluble *A. platensis* extract (Kwak et al., 2015). It has a maximal adsorption capacity of 98.5 percent against Chromium (Balaji et al., 2015). Therefore, Spirulina biomass is an excellent biosorbent for heavy metal removal.

Extraction of nutrients- Spirulina cultures have been employed to extract nutrients from wastewater generated by brine, olive oil mills, chicken litter, urban sewage, and sago starch industries (Markou et al., 2012; Wuang et al., 2016; Lababpour, 2017; Zhou et al., 2017). Spirulina eliminated 81.51 and 80.52 percent of nitrogen and phosphorus from municipal wastewater, respectively. Chemical oxygen demand in effluent from sago starch manufacturers and olive oil mills can be effectively removed with Spirulina (Phang et al., 2000; Markou et al., 2012). Spirulina can be used to purify aquaculture effluent, which is critical for high-density fish production.

Spirulina can remove 92.40 percent of ammonia and 50.4 percent of nitrate from wastewater produced by tanks cultivating

Indian main carps, Catla, Rohu, and Mrigal (Kamilya et al., 2006).

CONCLUSION

Spirulina is one of the most cost-effective nutritional supplements for fish farming. Its constituents accelerate growth, immunological responses, disease resistance, reproductive function, and pigmentation. Spirulina efficiently extracts nutrients from wastewater and can effectively eliminate heavy metals from aquaculture discharge. This water purification procedure not only lowers the cost of raw materials for growing Spirulina, which can be utilized as a dietary supplement in the aquaculture, but it also improves water quality and reduces water usage in high-density fish farms with restricted water exchange. Integrating Spirulina into traditional Recirculating Aquaculture Systems appears to be a great fish farming integrated technique.

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