

## Fermented soybean meal (FSFM) and *Spirulina* (*Arthrospira platensis*) biomass as potential substitutes for fishmeal for tilapia and other fish species cultivated in intensive systems

*Farelo de soja fermentado (FSFM) e biomassa de Spirulina (Arthrospira platensis) como potenciais substitutos à farinha de peixe para tilápias e outras espécies de peixes cultivadas em sistemas intensivos*

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### ABSTRACT

The constant and significant growth of aquaculture activity necessitates the intensification of cultivation systems and develop more efficient, cost-effective and bioavailable diets. In this context, the use of alternative ingredients to fishmeal, such as fermented soybean meal (FSFM) and *Spirulina* biomass (*Arthrospira platensis*), should be considered. This review article aims to conduct a survey of studies with alternative ingredients to fishmeal for tilapia and other fish species, cultivated in more intensive farming systems, such as the biofloc system. Thus, it can be concluded that these ingredients allow the replacement (partial or total) of fishmeal in diets for Nile tilapia and other fish species. Furthermore, important information about the benefits arising from intensive systems, benefits that justify their use in the cultivation of these animals, were also observed.

**KEYWORDS:** zootechnical performance; animal nutrition; animal health; biofloc system.

### RESUMO

O constante e expressivo crescimento da atividade aquícola, faz necessária a intensificação dos sistemas de cultivo e o desenvolvimento de dietas mais eficientes, baratas e biodisponíveis. Neste sentido, o uso de ingredientes alternativos à farinha de peixe, como o farelo de soja fermentado (FSFM) e a biomassa de *Spirulina* (*Arthrospira platensis*), devem ser considerados. Esse artigo de revisão tem por objetivo realizar um levantamento de estudos com ingredientes alternativos à farinha de peixe para tilápias e outras espécies de peixes, cultivadas em sistemas de cultivo mais intensivos, como o sistema de bioflocos, e os inúmeros benefícios desses ingredientes e sistemas intensivos para esses animais. Neste sentido, é possível concluir que esses ingredientes permitem a substituição (parcial ou total) da farinha de peixe em dietas para a tilápia-do-Nilo e outras espécies de peixes. Além disso, trazem informações importantes sobre os benefícios advindos de sistemas intensivos, benefícios que fundamentam seu uso no cultivo desses animais.

**PALAVRAS-CHAVE:** desempenho zootécnico; nutrição animal; saúde animal; sistema de bioflocos.

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### INTRODUCTION

In intensive fish production systems, feed costs can represent up to 60% of all expenses (BARBOSA et al. 2020). Fishmeal is a crucial ingredient in aquaculture diets because it is a protein with high biological value, and its exclusion from formulations can lead to a decline in zootechnical indices (GLENCROSS et al. 2011, TURCHINI et al. 2019). It is becoming less commercially available, and its cost remains high and constantly fluctuating (WORLD BANK 2020). An increase in animal costs results in higher expenses for aquatic animal production (EL BASUINI et al. 2016 and 2017, HOSSAIN et al. 2016). In this context, the search for alternative ingredients is a strategy for developing diets that reduce costs and optimize production performance (BULBUL et al. 2015, ZHANG et al. 2016, DANIEL 2017, ZHANG et al. 2021). Alternative ingredients are by-products or

more accessible products of plant or animal origin, derived from agricultural activities and/or processing industries (ABOWEI & EKUBO 2011).

Soybean meal is one of the most commonly used sources of vegetable protein because of its availability on the market and amino acid profile (SØRENSEN et al. 2009, DANIEL 2018). Complete replacement of fishmeal with this ingredient is not always possible due to its low palatability, high fiber content, and deficiency of some essential amino acids, which tend to limit the use of this meal (EL-DAHAR & EL-SHAZLY 1993, LIMA et al. 2014). Strategies such as microbial fermentation can improve amino acid profiles and nutrient availability, reduce fibrous components, and improve animal performance and health (HONG et al. 2004, SOTOUDEH et al. 2016, VAN NGUYEN et al. 2018). Fermentation is a bioconversion or metabolic degradation technique carried out by microorganisms (bacteria and/or fungi) that transform complex substrates into simple compounds (BALAKRISHNAN & PANDEY 1996). The preservation technique also improves the nutritional and functional properties of ingredients (ROSS et al. 2002, FRIAS et al. 2008). During the fermentation process, secondary metabolites with prebiotic action (antibiotics, low molecular weight bioactive peptides, enzymes and growth factors) are produced, along with an increase in the population of beneficial microorganisms with probiotic function (MUKHERJEE et al. 2015, DAWOOD & KOSHIO 2019, WANG et al. 2019).

It has been shown that fermented soybean meal (FSFM) can partially replace fishmeal in diets without compromising fish performance (CHOI et al. 2019, WANG et al. 2019, HE et al. 2020a,b). Numerous effects of FSFM on fish health have also been observed, including improvements in biochemical parameters (NOVRIADI et al. 2017 and 2018), lipid metabolism, total antioxidant capacity (JIANG et al. 2018), and intestinal health (WANG et al. 2016, LI et al. 2019, WANG et al. 2019). In addition, increased inflammatory responses and digestive enzyme activity (SHIU et al. 2015, SOTOUDEH et al. 2016, LI et al. 2020) were noted. The use of soybean meal as a substitute for fishmeal has been evaluated in tilapia diets, either partially (AL-KENAWY et al. 2008, LIN & LUO 2011, SHARMA & SAINI 2017) or completely (EL-SAYD & GABER 2002, AJANI et al. 2016).

Another alternative ingredient is the filamentous microalga *Spirulina (Arthrospira platensis)*, which is one of the main phytoplankton organisms studied for aquatic nutrition (AL-ZAYAT 2019, PLAZA et al. 2019, LEITE et al. 2019, SALAH EL-DIN et al. 2021). It is considered a rich source of easily digestible proteins and essential amino acids, as well as minerals, vitamins, fatty acids, and pigments (DE LARA ANDRADE et al. 2005). *Spirulina* is used in animal production as a source of protein and coloring (HABIB et al. 2008). In aquaculture, it has been evaluated as a dietary supplement (MANSOUR et al. 2021, SALAH EL-DIN et al. 2021, SOLIMAN et al. 2021) or as a partial or total substitute for fishmeal (CAO et al. 2018, ERDOGAN 2019, ROOHANI et al. 2019, RAJI et al. 2020), with no adverse effects on the health or performance of tilapia and several other fish species. *Based on this and the importance of Nile tilapia (Oreochromis niloticus) in national aquaculture, this literature review presents the main findings and improvements resulting from the use of fishmeal substitute ingredients, such as fermented soybean meal and Spirulina microalgae biomass, in the diet of Nile tilapia farmed in different intensive systems.*

## DEVELOPMENT

### National fish farming and fishmeal demand

According to data from the Brazilian Fish Farming Association, in 2023, Brazilian fish production increased by 3.1% compared to the previous year (860,355 tons), totaling 887,029 tons (PEIXE BR 2024). According to the Association, Nile tilapia is the most produced species, accounting for 579,000 tons, representing 65.3% of total fish production. An increase of 5.28% from the previous year (2022) and 103% from 10 years ago (2013). The national ranking of the largest tilapia producers began with Paraná, the largest producer, at 209,500 tons; followed by São Paulo at 75,700 tons; Minas Gerais at 58,200 tons; and finally Santa Catarina at 44,600 tons (PEIXE BR 2024).

By 2050, it is estimated that the world's population will reach 9.7 billion (UN 2021). With this increase in population, the demand for animal protein and more sustainable activities is also increasing. To meet these challenges, intensification of production systems, combined with the relentless search for more sustainable ingredients, is inevitable and necessary. Fish farming is a constantly expanding industry (FAO 2022 and 2024, PEIXE BR 2024), and the demand for sustainability in this sector depends on many factors, including lower feed costs. In intensive fish production systems, feed costs can reach 60% of the total expenses (BARBOSA et al. 2020).

Fishmeal is an important and commonly used ingredient in aquaculture diets. It is a protein of high biological value (above 55% crude protein - NRC 2011), and its exclusion from formulations can lead to a

decline in zootechnical indices (GLENCROSS et al. 2011, TURCHINI et al. 2019). In addition to its excellent amino acid profile, it also stands out for its fat-soluble and water-soluble vitamins and high levels of long-chain polyunsaturated fatty acids and minerals (MASUMOTO et al. 1996). As this ingredient becomes less commercially available, its cost, in addition to being high, remains in constant fluctuation (WORLD BANK 2020). This is due not only to increased demand from the feed industry but also to the strong fishing pressure on forage species, leading to the depletion of some of these stocks (NAYLOR et al. 2009). As cost rises, greater expenses will be incurred for the production of aquatic animals (EL BASUINI et al. 2016 and 2017, HOSSAIN et al. 2016). In recent years, there have been constant research efforts to replace fishmeal with alternative ingredients that reduce feed costs and optimize production performance (BULBUL et al. 2015, ZHANG et al. 2016, DANIEL 2018).

### Alternative ingredients to fishmeal

#### *Fermented soybean meal (FSFM)*

Soybean meal is one of the most commonly used sources of vegetable protein in animal nutrition due to its high protein content, balanced amino acid profile, and availability on the market (SØRENSEN et al. 2009; MUKHERJEE et al. 2016, SHARAWY et al. 2016, WANG et al. 2016, DANIEL 2018, JANNATHULLA et al. 2018). It is a product derived from soybean oil extraction and contains approximately 48% crude protein (MUKHERJEE et al. 2016).

The results recommend partially replacing fishmeal with soybean meal, ranging from 25% to 75%, for tilapia (AL-KENAWY et al. 2008, LIN & LUO 2011, AJANI et al. 2016, SHARMA & SAINI 2017). These findings allow plant protein sources to be incorporated into diets without compromising performance (EL-SAIDY & GABER 2002, AL-KENAWY et al. 2008, LIN & LUO 2011, AJANI et al. 2016, SHARMA & SAINI 2017). Improvements in nutrient use and animal health have also been observed (AJANI et al. 2016), as well as total production and net economic return (AL-KENAWY et al. 2008).

The total replacement of fishmeal is not always feasible (HANSEN et al. 2011, BONALDO et al. 2011, FERRARA et al. 2015). Despite its many qualities, soybean meal contains anti-nutrient factors (ANFs) (LIMA et al. 2014), including protease inhibitors, lectins, phytic acid, saponins, and fiber. In addition, it is less palatable and deficient in some essential amino acids (methionine and lysine) (EL-DAHAR & EL-SHAZLY 1993, LIMA et al. 2014), limiting the utilization of proteins and other nutrients by animals. Amino acid supplementation can enable complete supplementation (AJANI et al. 2016, EL-SAYD & GABER 2002), but some authors have reported a decrease in consumption (MAHMOUD et al. 2014). These challenges justify the application of the fermentation technique to this ingredient (JAKOBSEN et al. 2015, MONIRUZZAMAN et al. 2018, JANNATHULLA et al. 2019).

Fermentation is a food preservation and processing technique that also enhances the original nutritional and functional properties of foods (FRIAS et al. 2008). The process involves inoculating and providing ideal conditions for the growth of beneficial microorganisms (fungi and/or bacteria) in ingredients of animal or plant origin. The aim is to biologically convert complex substrates into simple compounds, forming typical fermentation products (carbon dioxide and alcohol) (BALAKRISHNAN & PANDEY 1996). The final product will also be rich in populations of probiotic microorganisms and secondary metabolites such as antibiotics, low-molecular-weight bioactive peptides, enzymes, and growth factors, all of which have prebiotic action (BALAKRISHNAN & PANDEY 1996, KADER et al. 2011, MUKHERJEE et al. 2015, DAWOOD & KOSHIO 2019, WANG et al. 2019).

For animal nutrition, the main fermentation technique used is solid-state fermentation (SSF) (YANG et al. 2021). This technique involves inoculating microorganisms into grains or bran using moisture levels between 40% and 80% (ORIOLE et al. 1988a,b, BORZANI et al. 1999, YANG et al. 2021) to produce fermented dry ingredients in the form of whole grains or powder (SUBRAMANIYAM & VIMALA 2012). *Aspergillus* spp., *Rhizopus* spp. and *Lactobacillus* spp. are the main microorganisms used in this method (SUBRAMANIYAM & VIMALA 2012) for the production of enzymes, organic acids, and biopesticide (LU et al. 1998, BATTAN et al. 2006). It can also be used to develop animal feed ingredients (MUKHERJEE et al. 2015, DAWOOD & KOSHIO 2019, WANG et al. 2019).

The main microorganisms used in the fermentation technique are from the *Bacillus* genus (WONGPUTTISIN et al. 2007, TENG et al. 2012), *Aspergillus* (ILYAS et al. 1995, HIRABAYASHI et al. 1998, HONG et al. 2004, KISHIDA et al. 2000, FENG et al. 2007a,b), *Lactobacillus* (AMADOU et al. 2010a,b, CHI & CHO 2016, WANG et al. 2016), and *Saccharomyces* (CHI & CHO 2016, HASSAAN et al. 2018). Each one is responsible for producing different products (lactic acid, ethanol, acetic acid, among others) because they respond differently to each substrate (NIBA et al. 2009). For example, *Lactobacillus* tends to produce lactic

and citric acid, while yeasts produce ethanol and CO<sub>2</sub> (SUBRAMANIAM & VIMALA 2012).

Studies have shown that fermentation increases the bioavailability and digestibility of nutrients while reducing anti-nutrient content in plant proteins (WANG et al. 2016, HASSAAN et al. 2018). In this fermentation process, the microorganisms involved in bioconversion degrade protein macromolecules into soluble low-molecular-weight compounds. These compounds, made up of small chains of amino acids and bioactive peptides, are better assimilated, improving the digestion and absorption of nutrients, as well as animal health (AZARM & LEE 2012, SANJUKTA & RAI 2016). In addition, other advantages and benefits of fermented soybean meal (FSFM) have been observed, including the following:

- Improved nutritional quality and antioxidant activity in soybean meal (DA SILVA et al. 2018);
- Source of enzymes, organic acids, and growth factors (KADER et al. 2011, MUKHERJEE et al. 2015, DAWOOD & KOSHIO 2019, HA et al. 2019, WANG et al. 2019);
- Increase in fibrinolytic enzyme activity, in vitro trypsin digestibility, and nitrogen solubility (MUKHERJEE et al. 2016, SHARAWY et al. 2016, WANG et al. 2016, JANNATHULLA et al. 2018);
- The crude protein content was increased by approximately 10% compared with the original ingredient without altering the essential amino acid profile (HONG et al. 2004);
- Decreases fiber content, ANF levels, and levels of toxic ingredients, facilitating absorption, use, and palatability of nutrients. Thus, improvements in feed efficiency and animal performance can be achieved (MAKKAR 1993, YAMAMOTO et al. 2010, JAKOBSEN et al. 2015);
- Immunostimulant action and tolerance to environmental stressors improve the health of farmed animals (MONIRUZZAMAN et al. 2018, JANNATHULLA et al. 2019).

FSFM is rich in bioactive peptides and can have a high protein and amino acid content and lower ANF and allergen content than soybean meal (SHIU et al. 2015, WANG et al. 2016, CHI & CHO 2016, LI et al. 2019). It is also rich in bioactive compounds with regulatory and functional roles, acting as an antihypertensive, antimicrobial, antioxidant, and antidiabetic agent (SINGH et al. 2014, SANJUKTA & RAI 2016).

FSFM has been widely tested as a fishmeal substitute and functional food in aquaculture diets. FSFM can replace 25%–60% of fishmeal in fish diets without compromising performance and, in addition, has numerous benefits for animal health, as shown in Table 1. Beneficial effects on fish health with the inclusion of FSFM in diets have also been reported: improved non-specific immune responses, reduced inflammation, modulation of the microbiota, and increased activity of digestive enzymes (YAMAMOTO et al. 2010, SHIU et al. 2015, LEE et al. 2016, ILHAM & FOTEDAR 2016, SOTOUDEH et al. 2016, NOVRIADI et al. 2017 and 2018, CHOI et al. 2019, RAHIMNEJAD et al. 2019). In addition, changes in biochemical and hematological parameters, lipid metabolism, hepatointestinal health, and total antioxidant capacity have also been observed (YAMAMOTO et al. 2010, AZARM & LEE 2012, BARNES et al. 2015, WANG et al. 2016, JIANG et al. 2018, NOVRIADI et al. 2018, WANG et al. 2019).

Table 1. Studies using fermented soybean meal (FSFM) from various fish species.

Tested parameters	Species/Weight (g)	Levels tested	Main results/Conclusion	Reference
Performance	Hybrid tilapia ( <i>Oreochromis niloticus</i> x <i>O. aureus</i> )/0.36	Control: No FP; Diet I: 0-20% FP or 57-31.6% DSBM; Diet II: 0-20% FP or 51-28.3% FSFM with encapsulated methionine	Lower performance on diets without PF. The addition of encapsulated methionine did not improve the growth of the group without FP.	WU et al., 2003
Performance, feed efficiency, digestibility, hematology, biliary status, and hepatointestinal morphology	Rainbow trout ( <i>Oncorhynchus mykiss</i> )/12.0	FP Diet; FS Diet; FSFM I diet: initial addition of 30% water (fermented for 7 h); FSFM II diet: 5% (10 h).	Better performance and feed efficiency in groups fed the FP and FSFM II diets; Better biliary and hepatointestinal health on fermented diets	YAMAMOTO et al. (2010)
Performance, biochemical parameters, and digestibility	Black snapper ( <i>Acanthopagrus schlegelii</i> )/1,17	FSFM (0, 80, 160, 240 and 320 g/kg) supplemented with methionine, lysine, and taurine to replace FP	Replacing FP with FSFM supplemented by up to 40% without compromising performance	AZARM & LEE, 2012
Performance, digestive enzyme activity, and changes in the	Grouper orange-spotted ( <i>Epinephelus coioides</i> )/4.46	Control with FP, replacement of FM by FS at 10%, 20 and 30% and at 10, 20, 30 and	Replacing FP with FSFM with up to 30% without compromising performance and feed efficiency. Hepatic,	SHIU et al. 2015

liver and distal intestine		40% by FSFM	intestinal, and digestive improvement after replacing FP with FS and FSFM	
Performance, organosomatic index, and intestinal histology	Trout strains <i>Shasta</i> x <i>McConaughy</i> ( <i>Oncorhynchus mykiss</i> )/23.4	Diets with 0, 35 or 50% FSFM and 40, 15 or 0% FP	Diets containing up to 35% FSFM and 15% FP are acceptable for both strains without compromising performance and intestinal histology.	BARNES et al. 2015
Performance, enzyme activity, and blood physiology	Barramundi ( <i>Lates calcarifer</i> ) / 5.04	FS or FSFM supplemented or not with organic selenium replacing FP	Performance improved with the use of organic selenium for FS and FSFM, but did not differ between the FP diet groups.	ILHAM & FOTEDAR, 2016
Performance, body composition, and digestive enzyme activity	Caspian brown trout ( <i>Salmo trutta caspius</i> )/2.1	FP replaced by FS or FSFM untreated or irradiated with $\gamma$ -radiation at 15 and 30 kGy	The gamma radiation level used was not efficient, but FS fermentation improved the final weight, proteolytic enzyme activity, and body composition.	SOTOUDEH et al. 2016
Dry matter and protein digestibility of FSFM diets compared with FS and FM	Grouper orange-spotted ( <i>Epimetheus coioides</i> )/22.01	Control with FP; Test diets with 300 g/kg FS or FSFM replacing FP.	Improved apparent digestibility of FSFM diets	ZHUO et al. (2016)
Performance, immune response, and intestinal morphology	Psetta maxima - turbot ( <i>Scophthalmus maximus</i> )/5.11	Control with PF; Test diets with PF replacement by FS or FSFM Lactobacillus plantarum at 15, 30, 45 and 60%	Replacement of FP protein by FSFM with <i>L. plantarum</i> by up to 45% without affecting the variables studied.	WANG et al. 2016
Performance, body composition, and enzymatic anti-oxidant activity	Rockfish ( <i>Sebastes schlegelii</i> )/1.2	FSFM (0, 8, 16, 24, 32%) replacing the FP protein with 0, 10, 20, 30 and 40%, approximately	Replacing FP protein with FSFM with up to 40% had no negative effect on animal performance.	LEE et al. 2016
Intestinal microbiota	Atlantic salmon ( <i>Salmo salar</i> )/50	Control with 30% FP, FS with 30% or FSFM with 30%	The use of FSFM promoted intestinal health and protection in salmon.	CATALÁN et al., 2017
Performance, fatty acid profile, and body composition	Japanese sea bass ( <i>Lateolabrax japonicus</i> )/13.3	FSFM (0, 25, 50 and 75%) replacing FP	Replacing FP with FSFM with up to 25% without compromising performance, meat quality, or feed use	LIANG et al., 2017
Performance and economic viability	<i>Anabas testudineus</i> /1,03	Control; FSFML replacing FP with 0, 25, 50, 75, or 100% of FP's PB	A performance similar to that of the control was observed up to an FSFML level of 50%. Economically, FSFML can be included in up to 50% of PF protein.	KADER et al. 2018
Performance, morphological condition, and serum biochemistry of the liver and distal intestine	Pompano ( <i>Trachinotus carolinus</i> )/17.01	FSFM (0, 25, 50, 75 and 100%) replaces the FS	Improvement in biochemical parameters according to the inclusion of FSFM. Improved intestinal health with the two highest contents of fermented foods	NOVRIADI et al. 2018
Performance,	Largemouth Bass ( <i>Micropterus</i> )	FSFMR (0, 20, 40 60 g/kg protein) replaced by	The best performance was	JIANG et al. 2018

body composition, and plasma biochemical parameters	<i>salmoides</i> )/17.1	FS	observed when substituting 40g/kg. Improved lipid metabolism and antioxidant capacity	
Performance, morphology, and intestinal microbiota	Yellow croaker ( <i>Larimichthys crocea</i> )/10.0	FSFM (0, 15, 30, 45, 60 and 75%) replacing FP	FSFM replacement of up to 45% of FP without negative effects on performance and intestinal integrity.	WANG et al. (2019)
Performance and feed use	Rainbow trout ( <i>Oncorhynchus mykiss</i> )	Control with FM; FSFM 1 and FSFM 2 (fermentation techniques), both with 20, 40 and 60% replacing FP.	Improvement in oxidative stress with the use of FSFM. Replacement of FP by up to 40% with fermented products with no effect on growth or feed use	CHOI et al. (2019)
Performance, ANF levels, digestibility, and intestinal health	Psetta maxima - turbot ( <i>Scophthalmus maximus</i> )/8,53	Control with FP (0%), 30%, 45% and 60% with FS and 30%, 45% and 60% with FSFM	Reduced ANF levels with fermentation, improved performance and digestibility of up to 45% of FSFM; improved intestinal health at the two highest levels	LI et al. (2019)
Performance, antioxidant status, intestinal morphology, and inflammatory response	Psetta maxima - turbot ( <i>Scophthalmus maximus</i> )/7,57	Control with FP; 45% FS or FSFM with <i>Enterococcus faecium</i> )	Fermentation neutralized the negative effects of FS, improved antioxidant capacity, suppressed inflammatory responses, and modulated intestinal microbiota	LI et al. (2020)
Performance, body composition, and biochemical and intestinal histology	Largemouth Bass ( <i>Micropterus salmoides</i> )/4.43	Control of 35 g/kg FP; Replacement of FP by FS and FSFM at 15%, 30%, 45%, and 60%	Replacing FSFM with up to 30% PF without compromising performance, digestibility, and intestinal health	HE et al. (2020a)
Performance, histology, and intestinal microbiota	Largemouth Bass ( <i>Micropterus salmoides</i> )/4.43	Control 35g/kg FP; Replacement of FP with FS or FSFM at 30% and 60%	Supplementation with FSFM of up to 30% of FP in diets without adverse effects on performance and intestinal health	HE et al. (2020b)
Performance, intestinal histology, enzyme activity, and expression of proinflammatory genes	Gentian grouper ( <i>Epinephelus fuscoguttatus</i> x <i>E. lanceolatus</i> )/9.0	FP control; Replacing FP with FSFM by 20% or 40%	Replacement with FSFM at 20-40% of FP affected performance, intestinal morphology, and expression of genes related to the immune system.	ZHANG et al. (2021)
Performance, carcass, and liver	Asian sea bass ( <i>Lateolabrax calcarifer</i> )/1.27	Control-unattractive FP; Control + FP with attractions; Replacement of FP mixtures of 20, 40, 60, 80, and 100% protein with 60% PBM and 40% FSFM	FSFM replacement for up to 60% of the PF without compromising performance	HONG et al. (2021)
Performance, microbiology, enzyme activity, and intestinal histology	South African catfish - jundiá ( <i>Rhamdia quelen</i> )	Control with FP; Replacing FP with FSFM <i>Lactobacillus acidophilus</i> at 7, 14, 21 and 28%	FSFM replacement by up to 21% without loss of performance. Lowest count of <i>Vibrionaceae</i> at 7-21%. FSFM did not affect the counts of heterotrophic bacteria, enzymatic activity, or intestinal histology.	DE OLIVEIRA et al. (2022)

Performance and intestinal health	<i>Nile tilapia</i> ( <i>Oreochromis niloticus</i> )	Plant-based diets including different levels (7%, 14%, 21% and 28%) of FSFM	The addition of 7% FSBM makes it possible to completely replace fishmeal without compromising fishmeal performance. Levels >21% of FSFM improved the intestinal health of tilapia.	PICOLI et al. 2022
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FP: Fish meal; FS: Soybean Meal; FSFM: Fermented soybean meal; DSBM: Hulled soy flour; PBM: Poultry by-product; Aa's: amino acids; kGy: kilo Gray; kDa: kilo Dalton; FSFMR: Fermented soybean meal residue; FSFML: Fermented with a mixture of soy flour and squid by-product; ANF's: Anti-nutritional factors.

#### Biomass of spirulina (*Arthrospira platensis*)

Unicellular proteins, including microalgae, yeasts, and bacteria, are promising alternative sources for replacing fishmeal in fish diets (HARDY 2010). They are often used as ingredients in aquatic organisms at different stages of development (ERDOGAN 2019, RAJI et al. 2020, MANSOUR et al. 2021). Among them, *spirulina* is one of the potential candidates for this substitution. It is a filamentous microalga with a smooth surface that contains phycocyanin and chlorophyll as its main photosynthetic pigments, which are responsible for its blue-green color (CAPELLI & CYSEWSKI 2010, ALI & SALEH 2012) (Figure 1).

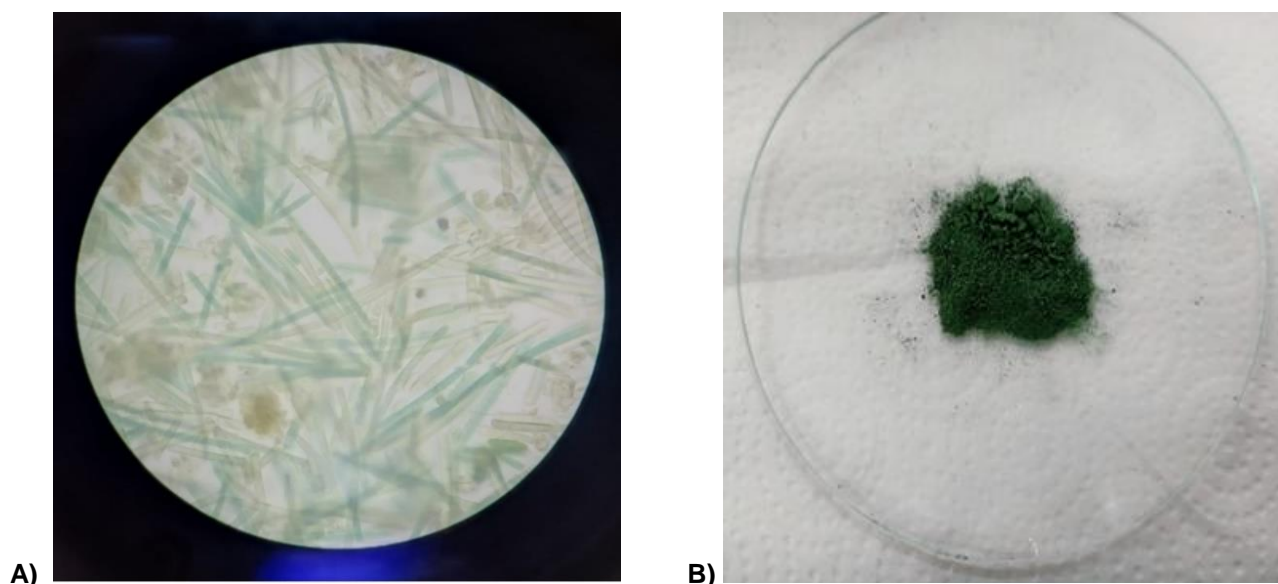


Figure 1. Biomass of *spirulina* (*A. platensis*) under (A) 40x optical microscopy (A) and in the wild (B).

*Spirulina* contains significant amounts of omega-6 polyunsaturated fatty acids, particularly gamma-linolenic acid and linoleic acid (HENRIKSON 1995, COLLA et al. 2004, BABADZHANOV et al. 2004). In addition, its composition includes high levels of iron, calcium, phosphorus, vitamins (vitamin E and B12), and pigments (phycocyanin, carotenoids and chlorophyll-a) (CAPELLI & CYSEWSKI 2010, ALI & SALEH 2012). Because of the absence of cellulose in the cell wall and the lack of phytate and oxalates, *spirulina* facilitates the assimilation of nutrients at the intestinal level (GUTIÉRREZ-SALMEÁN et al. 2015). It is a rich source of digestible protein (up to 70% of its protein content) and has a well-balanced amino acid profile (AL-DHABI & ARASU 2016, TOKU & ÜNAL 2003). *Spirulina* also plays a role in the treatment of various human pathological conditions, such as hepatotoxicity, cardiovascular diseases, cancer, and hyperlipidemia (HABIB et al. 2008, NAGAOKA et al. 2005). It also allows for greater inclusion in the substitution of various components in animal feed formulations (AHMADZADENIA et al. 2011, SIRAKOV et al. 2012).

In recent years, research has reported improvements in performance with the use of *spirulina* as a supplement or substitute for fishmeal in tilapia diets (Tables 2 and 3) (PLAZA et al. 2019, AL-ZAYAT 2019, ELABD et al. 2020, MANSOUR et al. 2021) and other fish species, such as the African catfish (*Clarias gariepinus*) (RAJI et al. 2018 and 2020), common carp (*Cyprinus carp*) (ABDULRAHMAN & AMEEN 2014), and brown trout (*Salmo trutta caspius*) (ROOHANI et al. 2019). In addition, benefits related to fish health, such as improved biochemical parameters (ABDEL-DAIM et al. 2020, MANSOUR et al. 2021, SOLIMAN et al. 2021), histological parameters (ABU-ELALA et al. 2016), and antioxidant capacity (AWED et al. 2020, ELABD et al. 2020, SALAH EL-DIN et al. 2021), are also noteworthy.



According to Tables 2 and 3, *spirulina* can be included in Tilapia diets as a supplement of up to 20%. As a substitute for fishmeal, up to 75% of the same species and for African catfish (*Clarias gariepinus*) (RAJI et al. 2018, ROSENAU et al. 2021), *common carp* (*Cyprinus carp*) (ABDULRAHMAN & AMEEN 2014), and brown trout (*Salmo trutta caspius*) (ROOHANI et al. 2019). *Complete replacement of fishmeal with spirulina biomass* (100%) has also been reported for Prussian carp (*Carassius auratus gibelio* var. CAS III) (CAO et al. 2018) and African catfish (RAJI et al. 2020), without harming the health or performance of these animals.

Table 2. *Studies using spirulina (Arthrospira platensis) as a supplement for Nile tilapia (Oreochromis niloticus).*

Tested parameters	Weight (g)	Levels tested as supplements	Main results/Conclusion	Reference
Performance, feed use, proximate body composition, and hemato-biochemical parameters	0.83	Control: Plant-based diet without SP; plant-based diets including 0.125, 0.25, 0.5, 0.75 and 1% SP	Supplementation of a plant-based diet with 0.5-0.65% SP improved performance, feed use, and blood biochemical parameters	MANSOUR et al. (2021)
Performance, feed use, and carcass composition	6.0	Control: DB without SP; diets containing 2.5, 0.5, 5 and 7.5% SP	Supplementation with 7.5% SP improved performance, protein use, and blood	AL-ZAYAT, 2019
Performance, hematological parameters, intestinal microbiota, and fillet quality	109.0	Control: DB without SP; Diet with 3% SP	Supplementation with 3% SP improved performance and fillet color without changing the intestinal microbiota. Increases in cortisol and triglyceride levels	PLAZA et al. (2019)
Performance and fillet composition	1.0	Control: DB without SP; Diet with 20% SP supplementation	Supplementation with 20% SP increased the protein level of the filets and kept performance (except S and CAA) of the control.	LEITE et al. (2019)
Reproductive parameters	55.0	Control: DB without SP; Diets including 5, 7.5, 10 and 20% SP	SP supplementation of 0.5-2% improved reproductive parameters	WAHB & SANGAK, 2017
Antioxidant capacity, DNA fragmentation, and protein fraction (gel electrophoresis - SDS-PAGE)	9.5	Control (T1): No exposure to EtBr and no SP; T2: EtBr 10 $\mu\text{g L}^{-1}$ ; T3: EtBr 10 $\mu\text{g L}^{-1}$ EtBr + 200 $\text{mg L}^{-1}$ SP; T4: EtBr 100 $\mu\text{g L}^{-1}$ ; T5: EtBr 100 $\mu\text{g L}^{-1}$ EtBr + 200 $\text{mg L}^{-1}$ SP	Supplementation with 200 $\text{mg L}^{-1}$ of SP protected against the negative effects on DNA and antioxidant capacity of tilapia exposed to ethidium bromide	SALAH EL-DIN et al. 2021
Biochemical, histopathological (brain, liver and kidneys), oxidative stress, and hematological indices	9.5	Control (T1): DB without exposure; T2: DB 15 $\text{mg/L}$ $\text{CuSO}_4$ ; T3: DN + 0.25% SP 15 $\text{mg/L}$ $\text{CuSO}_4$ ; T4: DB 15 $\text{mg/L}$ $\text{CuO-NPS}$ ; T5: DB + 0.25% SP 15 $\text{mg/L}$ $\text{CuO-NPS}$	Supplementation with 0.25% SP in fish exposed to $\text{CuSO}_4$ and $\text{CuO-NPs}$ protected against histopathological, biochemical, and hematological alterations and oxidative stress	SOLIMAN et al. (2021)
Biochemical, histopathological (brain, liver and kidneys), oxidative stress, and hematological indices	9.5	Control (T1): DB without exposure; T2: DB 15 $\text{mg/L}$ $\text{CuSO}_4$ ; T3: DN + 0.25% SP 15 $\text{mg/L}$ $\text{CuSO}_4$ ; T4: DB 15 $\text{mg/L}$ $\text{CuO-NPS}$ ; T5: DB + 0.25% SP 15 $\text{mg/L}$ $\text{CuO-NPS}$	Supplementation with 0.25% SP in fish exposed to $\text{CuSO}_4$ and $\text{CuO-NPs}$ protected against histopathological, biochemical, and hematological alterations and oxidative stress	SOLIMAN et al. (2021)
Performance and tissue antioxidant capacity	40.0	Control: DB; SS: 5.8 $\text{mg/L}$ intoxicated SS; SP: Diet with 1% SP; SP/SS: 1% spirulina diet and intoxicated with 5.8 $\text{mg/L}$ intoxicated SS	Supplementation with 1% SP did not alter the performance, but suppressed tissue oxidative stress induced by exposure to SS.	AWED et al. (2020)
Performance, digestive and biochemical enzymes, immune	26.0	Control: DB without SP; diets containing 0.25% or 0.5% SP	Supplementation with 0.25-0.5% SP nanoparticles improved performance,	ELABD et al. (2020)



status, antioxidants, resistance to <i>Aeromonas veronii</i> , and physical stressors			immune response, antioxidants, digestive enzymes, gene expression, and controlled infection	
Enzymatic and biochemical antioxidant biomarkers of lipid peroxidation	60.0	Control: no SP; 1% SP diet and no CPF exposure (15 g/L); diet with CPF exposure without SP; diet with CPF exposure 0.5%-1% SP	Supplementation with 1% SP improved enzymatic and biochemical antioxidant biomarkers and lipid peroxidation	ABDEL-DAIM et al., 2020
Performance and visceral indices, fillet composition, and texture	26.8	Control: DB; Diets 0.25% and 0.5% SP; Diets 0.25% and 0.5% <i>S. cerevisiae</i> ; Diets 0.25% and 0.5% <i>Rubrivivax gelatinosus</i>	Supplementation with 0.25-0.5% SP did not affect performance, increase nutritional quality, or preserve the texture characteristics of the filets.	GRASSI et al. (2020)
Final weight, histopathology, chromosomal abnormalities, DNA damage and biochemical-hematological parameters	100.0	Control: no SP and no exposure to CF; CF: DB + exposure to FC; FC+SP: DB + 0.5% SP + exposure to CF; SP: DB + 0.5% SP	Supplementation with 0.5% SP reduced the appearance of chromosomal abnormalities and DNA damage and improved erythrogram	MAHMOUD et al. (2019)
Markers of liver and kidney damage, serum biochemistry, and antioxidant status	60.0	Control: DB; SP1%: DB + 1% SP; DZN: DB + diazinon (0.28 mg/L); DZN-SP0.5: DB + DZN + 0.5% SP; DZN-SP1: DB + DZN + 1% SP.	Supplementation with 1% SP improved liver and kidney damage markers, serum biochemistry, and antioxidant status	ABDELKHALEK et al., 2017
Performance, body composition, and biochemical parameters	7.0	Control: DB without SP; Diet with 1% <i>A. fusiformis</i> ; Diet with 1% <i>A. platensis</i>	Supplementation with 1% SP ( <i>A. fusiformis</i> and <i>A. platensis</i> ) improved performance, body composition, and biochemical parameters	BELAL et al. (2012)

SP: Spirulina; FM: Fish meal; DB: Basal diet; S: Survival; AAC: Apparent feed conversion; CuSO<sub>4</sub>: copper sulfate; CuO-NPs: copper oxide; EtBr: ethidium bromide; FC: chemical products; CPF: chlorpyrifos; SS: sodium sulfate.

Table 1 Studies using spirulina (*Arthrospira platensis*) as a fishmeal substitute for tilapia and other fish species.

Tested parameters	Species/Weight (g)	Levels tested as a substitute	Main results/Conclusion	Reference
Productive performance, intestinal histomorphometry, plasma biochemistry and oxidative stress	Nile tilapia ( <i>Oreochromis niloticus</i> )/0.23	Control (0%): DB without SP 0% and 33% 66% 100% replacement of FM by SP	SP completely replaced FM without adverse effects on intestinal morphometry, protein metabolism and antioxidant response. Replacing 66% of FM with SP improved the production performance, regardless of the rearing system (clean water x BFT)	PICOLI et al. (2024)
Performance, carcass composition, and hematology	Red hybrid tilapia ( <i>Oreochromis niloticus</i> x <i>O. mossambicus</i> )/0.206	Control: DB with FM; Diets replacing PF with spirulina at 50, 75, 100%	The replacement of 75% of FM with spirulina did not differ from the FM control in terms of animal performance and carcass composition. Cost-effectiveness of spirulina-based diets compared with FM	EL-SHEEKH et al. (2014)
Organosomatic indices, feed use, carcass composition, and blood biochemical parameters	Nile tilapia ( <i>Oreochromis niloticus</i> )/0.89	Control: DB without spirulina; diets including 30, 45, 60 and 75% (0% FM) spirulina	Replacing 30% of FM with spirulina improved performance and organosomatic indexes, feed utilization, and carcass composition and reduced triglycerides compared with the control.	VELASQUEZ et al. (2015)

Performance, body and mineral composition, and hepatointestinal histology	Nile tilapia ( <i>Oreochromis niloticus</i> )/0.02	Control: Vegetable diet with corn gluten as a protein source; Seaweed diets 0, 25, 50, 75, and 100% gluten replacement; Diet with 100% <i>spirulina</i> to replace gluten	Replacing 100% corn gluten in vegetable diets with <i>spirulina</i> improves performance	HUSSEIN et al. 2013
Performance and obvious nutrient digestibility	African catfish - <i>catfish</i> ( <i>Clarias gariepinus</i> )/58.05	Control: DB with FM; Diet with 100% replacement of FM with SP; Diet with 100% replacement of PF with <i>Chlorella vulgaris</i>	Replacing 100% of the FM with SP improved the performance. Higher clear macronutrient digestibility coefficient	RAJI et al. (2020)
Performance, body composition, and sensory analysis	Common carp( <i>Cyprinus carp</i> )/32.7	Control: DB with FM; Diets with FM replacement by SP at 5, 10, 15 and 20%	Replacing 5-20% of FM with vitamin C SP+ improved weight gain and survival. Treatment with 10% <i>spirulina</i> resulted in the best sensory quality	ABDULRAHMAN E AMEEN, 2014
Performance, organosomatic index, body composition, and skin pigmentation	Malawi dolphin( <i>Cyrtocara moorii</i> )/3.15	Control: DB with FM; Diets with FM replacement by SP at 5, 10, 15 and 20%	Replacing 5%–15% of FM with SP improved performance and feed efficiency. The skin of animals fed 15% SP instead of FM showed greater pigmentation (carotenoids)	ERDOGAN 2019
Performance, hemato-biochemical, and antioxidant response	African catfish - <i>catfish</i> ( <i>Clarias gariepinus</i> )/42.07	Control: DB with FM; Diet with 50% and 75% replacement of FM; diet with 50% and 75% replacement of PF with <i>Chlorella vulgaris</i>	Replacing 75% of FM with SP improved performance and hemato-biochemical and antioxidant response	RAJI et al. 2018
Performance, meat quality, and fatty acid content	African catfish - <i>catfish</i> ( <i>Clarias gariepinus</i> )/50.0	Control: DB with FM; diet with 100% replacement of FM with SP.	Replacing 100% of FM with SP: harmed GDP, but FAC was not affected; altered visual characteristics and meat/skin quality; higher values/proportion of omega-3 and 6 FA	ROSENAU et al., 2021
Performance, body composition, fatty acid and amino acid profile, and pigmentation	Caspian brown trout (S <i>almo trout caspius</i> )/11.0	Control: DB with FM; diets replacing FM with SP at 2, 4, 6 and 8%	Replacing 8% of FM with SP improved performance, carcass composition, and pigmentation.	ROOHANI et al. (2019)
Performance, feed use, and digestive parameters	Prussian carp( <i>Carassis auratus gibelio</i> var. <i>CAS III</i> ) 5.0	Control: DB with FM; Diets replacing FM with SP at 50, 75 and 100%. Diets with 100% + 2% dicalcium phosphate and 100% + 0.28% crystalline lysine	The partial or total replacement (50, 75 or 100%) of FM with SP did not affect the performance. Total replacement improved diet digestibility and antioxidant capacity compared with the control.	CAO et al. 2018
Metabolic and growth responses	Zebrafish ( <i>Danio rerio</i> )/0.113	Six diets with SP inclusion levels of 0%, 2%, 4%, 6%, 8%, and 10% (SP0-SP10)	Supplementation with SP (6%) has substantial potential as a growth promoter, positively influencing lipid metabolism and antioxidant enzyme activity without compromising survival.	COLI et al. (2024)
Composition and apparent digestibility coefficients, essential macronutrients, energy, amino	Atlantic salmon( <i>Salmo salar</i> L.)/24.7	SP meal: 60% crude protein, 20 MJ kg <sup>-1</sup> crude energy, 16% nitrogen-free extract (NFE), 10% ash, 5% moisture, and 4% crude lipid.	SP meal as an alternative source of protein, rich in nutrients and low in anti-nutritional compounds and contaminants; diet containing 20% SP showed high digestibility of essential amino acids and long-chain n-3 polyunsaturated fatty acids	SEAN et al. (2023)

acids and fatty acids for spirulina flour.

Growth, nutritional value, and health status	<i>Micropterus salmoides</i> /13, 0	Control (SP0): 50% FM and no SP or PSP. The experimental diets contained 0.3% PSP, 3.0% SP (SP3 replacing 4.4% FM), and 6.0% SP (SP6 replacing 9.6% FM).	Replacing fishmeal with 3%–6% SP and adding 0.3% PSP can improve growth performance, protein efficiency, muscle nutritional value, liver health, antioxidant capacity, intestinal digestive enzyme activity, and intestinal microbiota.	ZHANG et al. (2024)
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SP: *Spirulina*; FM: Fish meal; DB: Basal diet; PSP: Purified polysaccharides from *S. platensis*; PIB: Weight gain; AAC: clear feed conversion.

### Biofloc system (BFT)

Biofloc cultivation or biofloc technology (BFT) is an intensive system with low water exchange achieved through the formation of a rich microbial community that cycles nutrients (EMERENCIANO et al. 2013). This system has great potential for aquaculture, as it makes it possible to increase aquaculture activity with less risk of disease and minimal environmental impact (MARISCAL-LAGARDA et al. 2012, BUHMANN et al. 2015, HU et al. 2015).

In the BFT system, the growth of specific microbial communities is stimulated through intense aeration and maintaining the correct carbon-nitrogen (C:N) ratio in the environment (AVNIMELECH 2007, EMERENCIANO et al. 2012). Various microorganisms are present in these flocs, predominantly bacteria (autotrophic and heterotrophic), but also yeasts, microalgae, ciliates, rotifers, and nematodes (MONROY-DOSTA et al. 2013). These microorganisms play crucial roles in water quality, recycling organic matter, and serving as food for cultivated aquatic organisms, while directly competing with other pathogenic microorganisms in the environment (AZIM & LITTLE 2008, EMERENCIANO et al. 2013 e 2017).

The main beneficial bacteria found in the BFT system are *Sphingomonas paucimobilis*, *Pseudomonas luteola*, *Pseudomonas mendocina*, *Micrococcus*, *Bacillus sp.*, *Nitrospira sp.*, and *Nitrobacter sp.* (MONROY-DOSTA et al. 2013). These dominant genera are associated with effective biocontrol of pathogenic microorganisms in the environment (HARGREAVES 2006, AVNIMELECH 1999 and 2009, CRAB et al. 2012). In addition, they produce rich microbial biomass (THOMPSON et al. 2002, MONROY-DOSTA et al. 2013), which can be used as food for cultivated species (MONROY-DOSTA et al. 2013, MARTÍNEZ-CÓRDOVA et al. 2015). Pathogenic bacteria of the *Aeromonas* and *Vibrio* genera tend to be found in the first few weeks of BFT cultivation and then stop multiplying (MONROY-DOSTA et al. 2013). This occurs because of competitive exclusion as the proliferation of beneficial bacteria increases (WU et al. 2012, MONROY-DOSTA et al. 2013).

Studies that allow the use of sub-optimal levels of protein for fish are essential for making the activity viable and assessing the potential of animals and systems (FURUYA et al. 2005, COSTA et al. 2009, AZIM & LITTLE 2008, DA SILVA et al. 2018, HISANO et al. 2019). The microbial biomass produced in this system can be used as a natural food source, providing up to 50% of the protein needs of tilapia (AVNIMELECH 2007). Consequently, it is possible to reduce crude protein in the diets of these animals by more than 30% without compromising performance (AZIM & LITTLE 2008). In addition, lower feed conversion rates compared to cultivation in clean water were also achieved (LUO et al. 2014).

### Growing tilapia in BFT

Tilapia are well adapted to this system, mainly because they are filter feeders, robust and fast-growing (AVNIMELECH 2011 and 2015). They can feed on the flakes present in the culture medium, regardless of the size of the aggregate (EKASARI 2014). These flakes are rich in protein, ranging from 24% to 32% (AZIM & LITTLE 2008, ELÍAS et al. 2015, LIMA et al. 2018). BFT technology also allows tilapia to be farmed at high densities and high yields (10 to 40 kg/m<sup>3</sup>), even compared to clear water farming (EKASARI 2014).

An increase in density from 15 to 45 tilapia/m<sup>3</sup> during the fattening phase of BFT resulted in higher productivity (LIMA et al. 2015), similar to that observed by WIDANARNI et al. (2012). It is believed that BFT farming can also improve the health of tilapia because the flakes contain various bioactive compounds, including essential fatty acids, carotenoids, free amino acids, and chlorophylls (JU et al. 2008), minerals (TACON et al. 2002), and vitamin C (CRAB et al. 2012). All of these compounds have positive effects on aquatic organisms, such as increasing their antioxidant status, growth, reproduction, and immune response in animals (TACON et al. 2002, JU et al. 2008, CRAB et al. 2012).

Animals raised in this system can also benefit from the reduced presence of exogenous pathogens

because it is a closed system. Some studies have shown that potentially pathogenic bacteria, such as *V. harveyi*, can be reduced in BFT under suitable cultivation conditions (CRAB et al. 2010 and 2012, ZHAO et al. 2012).

## CONCLUSION

The search for greater efficiency and sustainability in aquaculture has made it essential to search for alternative ingredients to fishmeal, as well as more intensive and biosecure farming systems. *In this context, this literature review highlights the significant potential of ingredients such as fermented soybean meal (FSBM) and spirulina biomass (Arthrospira platensis) in aquaculture diets.*

It can be concluded that these ingredients can be partially or completely substituted for fishmeal in diets for Nile tilapia and other fish species, offering numerous benefits in terms of performance and health parameters. In addition, valuable information on intensive systems, such as the biofloc system, was highlighted. These benefits provide a solid basis for its use in the cultivation of these animals.

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