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Review

Spent oyster mushroom substrate as a potential bioactive and nutritional food ingredient for tilapia culture: a review

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ABSTRACT. Nile tilapia (*Oreochromis niloticus*) is one of the most economically important fish cultivated in intensive aquaculture; however, these conditions cause stress and reduce its immune system responses, making it susceptible to infections and reducing production yields. Spent oyster mushroom substrate (SOMS) is the residue left after *Pleurotus* spp. mushroom cultivation, and it is expected that for every kilogram of harvested mushrooms, 5 kg of residue is generated. However, during solid fermentation of the used substrate, mycelium can change substrate composition by degrading lignocellulosic material, making it more digestible and enhancing its chemical composition. Thus, it becomes a novel raw material for aquaculture; mycelium is rich in bioactive compounds such as antioxidants and polysaccharides, which can reduce stress and even act as immunostimulants. SOMS and its effect on Nile tilapia have recently become a focus due to its benefits in health promotion and growth performance. In this review, we will explore its composition and uses in aquaculture.

Keywords: *Oreochromis niloticus*; *Pleurotus*; mushroom by-products; immunostimulants; antioxidants; β-glucan

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is recognized as the third most cultivated finfish under intensive aquaculture worldwide, with 4,407,200 t in 2020, behind silver carp (*Hypophthalmichthys molitrix*) and grass carp (*Ctenopharyngodon idella*), representing 9% of total production, mainly because of its short production periods, which makes it an essential and cheap protein source in underdeveloped countries (FAO 2022). However, the growing demand for aquaculture feed at low cost derived from the intensification of fish culture exposes the organisms to multiple stress factors that generate cellular damages and physiological disorders, reducing survival rate due to immunological suppression and exposing the organism to infectious diseases (Armenta-López et al. 2015, Mahdy et al. 2022).

Nowadays, the primary strategy to control diseases or to reduce oxidative stress is the use of vaccines and antibiotics; however, it has restrictions, and indiscriminate use may cause long-term problems such as resistant bacteria (Heuer et al. 2009, Banerjee & Ray 2017). Alternatively, nutrition-focused health is an investigation line that searches for organisms' well-being through their intestinal health by implementing diets that stimulate intestinal microbiota, thus promoting immune and antioxidant responses to increase survival rate (Makled et al. 2017, Lizárraga-Velázquez et al. 2018, Galeana-López et al. 2020, Flores-Méndez et al. 2022).

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Yeast has long been used in aquaculture as an alternative protein source and bioactive ingredient because of its cell wall composition and accessibility (Mahdy et al. 2022). However, new tendencies in the search for bioactive molecules by taking advantage of raw materials with no commercial worth, such as agricultural and agro-industrial wastes with the potential to be used in areas such as aquaculture, is a way to give added value to a residue and reduce its ecological impact.

Edible mushrooms have been a broad family of fungi consumed since ancient times mainly because of their culinary value and vast quantity of compounds associated with medicinal properties (Aida et al. 2009). Oyster mushrooms (*Pleurotus* spp.) are the second most cultivated mushrooms worldwide mainly because of their easy handling and capacity to grow on lignocellulosic materials such as agro-wastes (Sánchez 2010). However, it is reported that for every kilogram of freshly produced mushrooms, 5 kg of by-products are generated, the named spent oyster mushroom substrate (SOMS), leftovers of the digested substrate, and mycelium; thus, proper waste management is needed to avoid ecological hazards (Leong et al. 2022). Many efforts are made to find the best way to dispose of this waste, such as energy, compost, and animal feed, especially livestock (Hanafi et al. 2018). Nevertheless, its use as an element to promote aquaculture is still a matter of study.

In this review, we will address the potential use of SOMS as aquafeed, considering it not only as a waste but as an additive with functional qualities to promote fish well-being, giving it added value.

Spent oyster mushroom substrate (SOMS) production

Oyster mushrooms are organisms known as white rot fungi, which can grow on a vast quantity of substrates rich in lignin-cellulose as mycelium can secrete digestive enzymes that break down those complex molecules (Mahari et al. 2020), making it highly adaptable to different materials, like agricultural and agro-industrial wastes, and easy to grow. The oyster mushroom is the third most cultivated edible mushroom worldwide. Asian countries are the primary producers; China is the leading grower, with 87% of the total world production of this species (6 billion kilograms in 2013), highlighting two: *Pleurotus ostreatus* and *P. cornucopiae* (Royse et al. 2017). Edible mushrooms are attractive for their nutritional value, contain 5-15% dry matter, are a good source of dietary protein, are low in fat, and are rich in fiber (Grimm & Wösten 2018, Rathod et al. 2021).

After harvesting the mushrooms, the used substrate can no longer be used for mushroom production since the fungus has already been used to develop, and the mycelium is weakened; thus, it is considered waste. That waste is named SOMS, which consists of residual mushroom mycelia, substrate leftover, extracellular enzymes, and inorganic nutrients (Lou et al. 2017). This waste is highly available since it is considered that for every kilogram of harvested mushrooms, 5 kg of SOMS is produced (Patel et al. 2012); considering an industrial production of tons, it represents a disturbing ecological hazard if not managed correctly.

Edible mushrooms like *Pleurotus* spp. are excellent since they have a unique way of turning fibrous material into a good quality protein food; that is how it gained its name as "forest meat", it is not from an animal source, but has the same quality and can be found anywhere (Dimitrijevic et al. 2018). Aside its nutritional value, oyster mushrooms are known for their medicinal properties because of the presence of secondary metabolites that develop during mushroom production, such as polysaccharides and bioactive compounds like terpenes, fatty acids, and polyphenols, which are associated with health promotion (Patel et al. 2012, Golak-Siwulska et al. 2018). These compounds are not only present in fruiting bodies but also in the mycelium. Thus, SOMS can become an economical ingredient with high nutritional value and bioactive properties that can be used in aquaculture, giving it added value and promoting a circular economy (Fig. 1). Studies have investigated different uses of SOMS for animal feed, such as poultry (Hassan et al. 2020) and ruminants (Mhlongo et al. 2021), but aquafeed uses are less investigated. We will discuss their variable composition to explore how SOMS can be an additive or ingredient for aquafeed.

Chemical composition

As earlier explained, the substrate is media-rich in lignin and cellulose, where oyster mushrooms grow. Although *Pleurotus* spp. can grow on comprehensive lignin-cellulose materials, its composition may vary due to the chemical nature of the selected substrate, which affects the spent substrate as well. The Table 1 summarizes different compositions of spent mushroom substrates comparing *Pleurotus* strains and used substrates. Selection of a suitable substrate is crucial to improve mushroom productivity (Rodrigues et al. 2012), and the understanding of substrate changes during solid fermentation is a must to comprehend its needs during and after the mushroom's solid fermentation to ensure significant production yields and its

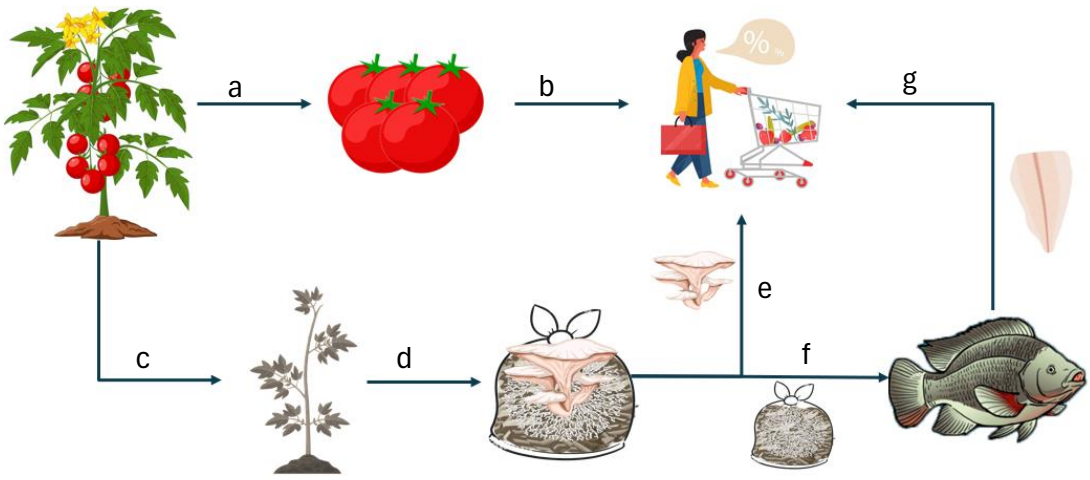

Figure 1. Chain value was added using tomato as an example. a) The harvested fruit is intended for b) human consumption while c) leftover plant biomass can be used as d) substrate for oyster mushroom cultivation, and e) harvested mushrooms are intended for human consumption while f) spent oyster mushroom substrate can be used as a bioactive additive ingredient to f) improve fish production in aquaculture to g) produce good quality and economical protein intended for human consumption.

relation with fruiting bodies size. However, studies concerning *Pleurotus* cultivation focus on production by testing different substrates and their initial composition with mushroom production, which complicates data compiling SOMS composition.

One of the main changes associated with solid fermentation by *Pleurotus* is a reduction of carbohydrates and fibrous content (crude fiber, cellulose, hemicellulose, and lignin) and an enhancement in the protein, fat, and ash content of SOMS (Nur et al. 2015).

The biochemical composition of the SOMS may depend on the substrate composition and strain used. Nur et al. (2015) compared changes in Malaysia's most common substrates used for mushroom production, finding that the most significant change is the enhancement of protein content after the cultivation cycle. Compared to the initial substrate, SOMS had more than 2,000% increase in protein content, from 0.54 to 14.5% for *P. sajor-caju* and 16.1% for *P. ostreatus*, which resulted in an incredible way of turning a lignocellulosic material into good quality protein material and reducing nearly 30% of total carbohydrates, which makes it a potential ingredient that can be used as a protein source since *Pleurotus* protein content and quality is comparable, and even superior, to those of eggs, milk and meat, due to its good distribution of essential and non-essential amino acids (Diamantopoulou et al. 2023). However, Sánchez et al. (2008) evaluated changes in substrate composition (tomato stubble, by itself and mixed with vineyard pruning or wheat straw) of spent mushroom substrate after production by using two *Pleurotus* strains, namely *P. pulmonarius* (IE-4) and *P. ostreatus* (IE-8). After harvest, the main reduction was found in protein content, contrary to the study realized by Nur et al. (2015), which had an exorbitant increase; in this study, nearly 50% of substrate protein was used by the mushroom to develop as nitrogen is a vital macromolecule needed for fruiting bodies formation substrates with higher protein content showed better fruiting bodies yields. Ether extract also showed a reduction of 33 to 69%; authors discuss that mushrooms use fats during their metabolism and fruiting body formation, contrary to Nur (2015), who reported an enhancement of 230% in fat in spent mushroom substrate. Finally, Sánchez et al. (2008) report that total mineral content tends to either increase when organic matter is consumed in major proportion or decrease when minerals are assimilated during mushroom development and fructification.

The most crucial change in SOMS after mushroom production is undoubtedly the degradation of lignocellulosic material since *Pleurotus* spp. can produce different amounts of lignocellulosic enzymes to adapt and grow in the substrate (Rodrigues et al. 2012), central enzymatic systems are hydrolytic enzymes namely lignin peroxidase, laccase and manganese-dependent peroxidase, which allows polysaccharide

Table 1. Proximal composition (%) of spent oyster mushroom substrate of different *Pleurotus* strains. NS: not specified. TP: total protein. TF: total fat. TC: total carbohydrates. CF: crude fiber. NDF: neutral detergent fiber. ADF: acid detergent fiber. REF: reference.

Strain	Substrate	Cycle of cultivation	TP	TF	TC	CF	NDF	ADF	Ash	REF
P. ostreatus	Rubber sawdust	6-7 weeks	16.1	23.78	63.57	NS	NS	NS	5.299	Nur et al. (2015)
	Rice straw	NS	6.00	2.80	NS	21.95	NS	NS	31.85	Faiza et al. (2020)
	Red grape pomace	28 days	12.69	NS	NS	NS	61.54	55.11	NS	Hassan et al. (2020)
	Corn straw	NS	4.80	NS	NS	NS	60.37	NS	7.90	Ramírez-Bibriesca et al. (2010)
	Sawdust	4 weeks	7.88	1.71	NS	29.57	NS	NS	9.92	Foluke et al. (2014)
	Corn cob, beet pulp, kapok meal	NS	7.9	0.3	NS	24.2	74.8	49.4	4.4	Back et al. (2017)
	Cotton waste	NS	6.9	0.9	NS	NS	61.8	59.3	10.8	Kwak et al. (2009)
	Tomato stubble	40 days	6.9	NS	73.8	NS	NS	NS	13.5	Sánchez et al. (2008)
	Tomato stubble + vineyard pruning	40 days	7.6	NS	72.5	NS	NS	NS	12.9	
	Wheat straw + tomato stubble	40 days	6.0	NS	73.2	NS	NS	NS	14.5	
	Wheat straw	30 days after harvest	5.41	NS	NS	NS	NS	NS	20.5	Owaid et al. (2017)
	70% wheat straw, 20% sawdust and 10% date palm fibers	30 days after harvest	6.24	NS	NS	NS	NS	NS	19.0	
	50 %Wheat straw, 30% sawdust and 20% date palm fibers	30 days after harvest	5.82	NS	NS	NS	NS	NS	17.5	
	Wheat straw	30 days after harvest	6.24	NS	NS	NS	NS	NS	16.5	
	70% wheat straw, 20% sawdust and 10% date palm fibers	30 days after harvest	5.41	NS	NS	NS	NS	NS	14.0	
	50 %Wheat straw, 30% sawdust and 20% date palm fibers	30 days after harvest	6.24	NS	NS	NS	NS	NS	12.5	
P. pulmonarious	Tomato stubble	72 days	8.5	0.3	72.2	NS	NS	NS	12.4	Sánchez et al (2008)

Continuation

P. pulmonarious	Tomato stubble, vineyard pruning	72 days	7.3	0.4	72.1	NS	NS	NS	13.9	Sánchez et al (2008)
	Wheat straw, tomato stubble	72 days	6.4	1.2	67.5	NS	NS	NS	18.2	
P. sajor-caju	Rubber sawdust	6-7 weeks	14.5	23.22	61.45	NS	NS	NS	5.146	Nur et al. (2015)
	Rice straw	8 weeks	9.28	0.8	NS	NS	60.29	52.3	15.49	Fan et al. (2022)
P. eryngii	Corn, wheat bran, and brewer's grain	NS	8.46	2.09	NS	5.27	NS	NS	4.58	Park et al. (2012)
	Sawdust, rice bran, and corn cobs	NS	5.9	1.4	NS	NS	76.1	59.1	9.2	Kwak et al. (2008)
	Sawdust, rice bran, and corn cobs + LAB fermentation	NS	5.7	1.4	NS	NS	78.8	66.0	6	Kim et al. (2012)
P. cornucopiae	Wheat straw	30 days after harvest	4.58	NS	NS	NS	NS	NS	18.5	Owaid et al. (2017)
	70 %Wheat straw, 20% sawdust and 10% date palm fibers	30 days after harvest	4.99	NS	NS	NS	NS	NS	21	
	50 %Wheat straw, 30% sawdust and 20% date palm fibers	30 days after harvest	5.19	NS	NS	NS	NS	NS	14	
P. salmoneos-tramineus	Wheat straw	30 days after harvest	6.24	NS	NS	NS	NS	NS	16	Owaid et al. (2017)
	70 %Wheat straw, 20% sawdust and 10% date palm fibers	30 days after harvest	4.58	NS	NS	NS	NS	NS	13	
	50 %Wheat straw, 30% sawdust and 20% date palm fibers	30 days after harvest	4.16	NS	NS	NS	NS	NS	13	

degradation so mushrooms can access to carbohydrates as a nutrient for growth and fructification (Bushwell et al. 1996). This last feature makes it a promising and viable ingredient for animal feed since mushroom fermentation makes it a more digestible material, as *Pleurotus* mushrooms have high selectivity on lignin and hemicellulose degradation (Ramírez-Bribiesca et al. 2010). Its good protein content also positions it as a potential additive for tilapia culture.

Bioactive compounds in SOMS

Mushrooms, in general, have been focused mainly because of medicinal attributes associated with their broad synthesis of secondary metabolites with bioactive activity promoting animal health benefits. Oyster mushrooms, as well, are appreciated because of bioactive molecules such as phenolics, pigments, polysaccharides, and terpenoids, which are related to immune system enhancement, act as metabolic active-

tors, have antimicrobial and antioxidant capacity with rejuvenating and energy booster activity (Patel et al. 2012, Özdal et al. 2019). These compounds are mainly associated with the fruiting body of the edible mushroom; however, mycelium is rich in them as well (Özdal et al. 2019); as SOMS is composed of fermented substrate and mycelium, it can serve as a biofabric for the extraction of secondary metabolites with bioactive compounds, but it is essential to highlight that oyster mushroom composition depends on the strain, substrate, and culture conditions (Lavega et al. 2023). It is essential to evaluate the changes in bioactive compound content in SOMS during fungal substrate fermentation to determine if it can be used as a relevant or viable source of bioactive compounds, and these evaluations have not been explored in *Pleurotus* cultivation papers. Besides, SOMS is considered an agro-industrial waste with no worth, so its use for bioactive compound extraction could generate additional profits for mushroom producers. So, selecting the most adequate extraction method, according to the focused molecule properties, is also necessary to ensure full exploitation of this raw material. Lizarraga-Velázquez et al. (2020) summarized the main extraction techniques that can be used for bioactive compounds extraction in plant wastes, including its pros and cons, listing maceration (with the use of solvents), hydrodistillation (extraction with water), enzyme-aided extraction, ultrasonic and microwave-assisted extraction, pressurized liquid extraction and supercritical fluid extraction. In this review, they concluded that, as the need for bioactive molecules increases, the extraction process's optimization must focus on reducing environmental impact, obtaining high-quality extracts and safe products, reducing energy and solvent consumption, and increasing yields of the final products. Though they are focused on plant residues, extraction methods can also be translated to mushroom by-products, SOMS included.

Antioxidant compounds

The antioxidant activity associated with oyster mushrooms is attributed to their content of phenolic compounds, flavonoids, and alkaloids (Nuhu et al. 2011, Morris et al. 2017). These molecules can exhibit both antimicrobial and antioxidant activity, the former preventing the development of pathogenic bacteria and the latter inhibiting free radicals to avoid cellular damage.

Pleurotus spp. extracts from mycelium have been studied for a while due to their antibacterial activity against different pathogenic microorganisms. Özdal et al. (2019) proved a hot water extract of three different oyster mushroom species and found them effective in inhibiting *Xanthomonas campestris*, *Arthobacter agilis*, *Helicobacter pylori*, and *Klebsiella oxycota*. Morris et al. (2017) evaluated the antimicrobial activity of a hot water extract from *Pleurotus* mycelium. They found it could cause autolysis of microbial strains such as *Candida intermedia* and *Bacillus subtilis*. The antimicrobial effects of oyster mushroom extracts may depend on the solvent used; an ethanolic extract tends to contain more bioactive metabolites due to its affinity for many phenolic compounds due to its polarity. However, hot water extracts are also effective in their antimicrobial effect when the same nitrogen source is used in the mycelium media. This last finding is important because the antimicrobial attributes of *Pleurotus* extracts also depend on the mycelium culture media. Vamanu (2012) compared the effect of different nitrogen sources in the liquid culture to grow mycelium with significative antimicrobial activity. In this study, ammonium sulfate resulted as the treatment with the most antimicrobial effect, being effective against strains of *Bacillus cereus*, *Listeria innocua*, *Candida albicans*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

The imbalance between oxidants and antioxidants, named oxidative stress, generates reactive oxygen species (ROS), which can alter biomolecules and cause disease. Antioxidants impede oxidation, reduce the effects of ROS, and restore balance (Sies et al. 2017, Pisoschi et al. 2021). Several publications state the antioxidant potential of oyster mushroom fruiting bodies; however, its by-products, such as spent substrate with abundant mycelium, are less studied but as rich as the fruiting body in those compounds (Zhang et al. 2016). Lavega et al. (2023) compared mushroom fruiting bodies, mycelium, and spent substrates to evaluate *Pleurotus* spp. antioxidant activity. Results are summarized in Table 2.

In the case of phenolic compounds, although they are not specified for two species, *P. ferulae*, and other evaluated mushrooms, spent substrate showed a significant quantity of those compounds compared to pure mycelium and fruiting body, suggesting that phenolic compounds are accumulated in the substrate from the degradation of phenolic products such as lignin (Kwak et al. 2015). It is essential to evaluate the antioxidant activity of the three sources to evaluate their possible uses. The antioxidant power of different mushrooms is mainly associated with and evaluated in polysaccharide extracts, which are hydrosoluble com-

Table 2. Total phenolics and antioxidant activities in fruiting bodies, mycelium, and spent substrate of different *Pleurotus* species. SOMS: spent oyster mushroom substrate.

		Strain	Source		
			Fruiting body	Mycelium	SOMS
Total phenolic compounds (mg GAE g extract^{-1})		*P. ferulae*	3.29	1.33	9.64
		P. citrinopileatus	6.96	3.22	-
		P. ostreatus	2.64	2.13	-
Antioxidant activity	DPPH (IC50 mg^{-1} mL^{-1})	*P. ferulae*	1.76	8.19	0.48
		P. citrinopileatus	0.47	5.47	1.87
		P. ostreatus	2.43	6.47	-
	ABTS (IC50 mg^{-1} mL^{-1})	*P. ferulae*	10.26	14.42	6.75
		P. citrinopileatus	3.83	13.47	7
		P. ostreatus	8.17	9.90	-
	FRAP (mM TEAC g^{-1} extract^{-1})	*P. ferulae*	15.6	7.25	246.08
		P. citrinopileatus	36.64	10.17	49.25
		P. ostreatus	4.36	10.56	-

pounds (Zhang et al. 2016). There is an area of great opportunity to explore the antioxidant activity of extracts and by-products of *Pleurotus* spp. and the identification of the extracted compounds.

As aquaculture is a growing sector in need of finding low-cost natural compounds as food additives that can enhance the well-being of farmed organisms, in our group work, we have experimented with the uses of unconventional additives such as agricultural and agro-industrial wastes. Galeana-López et al. (2020) used phenolic compounds extracted from corn husks as additives incorporated in Nile tilapia diets in 100 and 200 mg of phenolic compounds to evaluate its effects on the growth performances of fish. Their results showed that as the inclusion of phenolic compounds increases, so do growth performance parameters such as final weight, weight gain, and specific growth rates, suggesting that using phenolic compounds may improve growth performances in Nile tilapia. In a later publication, Galeana-López et al. (2021) tested a diet for Nile tilapia under low oxygen conditions, with the addition of corn husk meal in a concentration of 25 g kg^{-1} of diet to ensure an amount of 0.28 g Gallic acid equivalent (GAE) kg^{-1} to evaluate its effects. Their results found that fishes under hypoxia conditions and fed with the experimental diet promoted antioxidant responses in Nile tilapia through catalase hepatic antioxidant response. This last publication is important because it demonstrates that if a complex matrix such as corn husk meals can be used as a source of phenolic compounds, SOMS can also be used. According to Table 2, the only reported composition of phenolic compounds in SOMS is 9.64 mg GAE g^{-1}, which can be translated to 964 mg GAE 100 g^{-1}, while Galeana-López et al. (2020) report an amount of 171.33 mg GAE 100 g^{-1}of corn husk meal, which means that SOMS is more than twice rich in phenolic compounds, so to ensure the same concentration less amounts of SOMS meal might be used. So experimental analysis must be done to determine if phenolic compounds found in SOMS can be as effective as those found in corn husk meal, which is reported to be mainly chlorogenic acid, ferulic acid, p-coumaric acid, and p-hydroxybenzoic acid (Galeana-López et al. 2021).

Polysaccharides in SOMS

Fungal polysaccharides are bioactive fibers that have gained significant attention since they have serial beneficial properties such as antioxidant, antiviral, antitumor, and immunomodulatory effects (Zhao et al. 2023). These compounds can include polysaccharide-protein complexes, polysaccharide-peptides, and β-glucans. These last molecules are significant constituents of some plants, yeast, and mushroom cell walls, which are polymers composed of glucose molecules bonded by glycosidic bonds whose structural composition can differ depending on the source. For example, the ones from oats and barleys are linked by β-1,3 and β-1,4 glycosidic bonds, while the ones coming from yeast and mushrooms are linked by β-1,3 and β-1,6 (Ching et al. 2021). The most common sources of β-glucans used commercially come from yeast, specifically *Saccharomyces cerevisiae*. Its cell wall comprises 55-65% β-glucans, immunomodulators

targeting innate immune responses (Novak & Vetvika 2008). These compounds constitute the fungal cell wall and are responsible for their medicinal attributes; however, concentration varies among species, cultivars, growing environments, drying conditions, and isolation methods (Cerletti et al. 2021). Table 3 summarizes the β-glucan content in different *Pleurotus* species fruiting bodies (Sari et al. 2017).

Although the fruiting body of oyster mushrooms is the main protagonist of different studies, recently, given the increasing demand for these edible mushrooms, by-products have gained attention. During mushroom production, a non-negligible number of "wastes" is generated, listing noncommercial fruiting bodies (in terms of quality standards), stipes, and spent mushroom substrate (Antunes et al. 2020). SOMS mainly comprises substrate leftovers and mycelium, which contains β-glucan but in less quantity than the fruiting body. Nitschke et al. (2011) evaluated β-glucan composition in several mushroom mycelia (grown in liquid culture) and fruiting bodies, including *Pleurotus* species, namely *P. ostreatus*, *P. pulmonarious* and *P. eryngii*. Their results quantified a total of 2.97, 0.41, and 1.33 g 100 g^{-1} in mycelia while calculated fruiting bodies of *P. ostreatus* and *P. eryngii* were 8.29 and 12.91 g 100 g^{-1}. According to the authors, these high differences between mushroom fruiting bodies and mycelia are mainly associated with the formers needing a more solid cell wall because of their compact nature.

In contrast, mycelia need to be more flexible for nutrient-uptake purposes. Nevertheless, the quantification of β-glucan in the spent mushroom substrate is poorly explored, representing a missed opportunity to access and obtain valuable bioactive compounds. Chirapongsatonkul et al. (2019) extracted a crude glucan extract from *Schizophyllum commune* spent mushroom substrate with pressurized hot water extraction and were able to obtain 26.67 g 100 g^{-1} of β-glucans, a higher amount than the highest reported in *P. ostreatus* fruiting body in Table 3 (24.2 g 100 g^{-1}). However, it is a different mushroom species, and it represents a possible way of obtaining a good amount of β-glucans from a no-worth residue like spent mushroom substrate.

Besides, an investigation made by Park et al. (2012) evaluated the use of SOMS from *P. eryngii*, as a whole matrix, in diets for elk (*Cervus elaphus canadensis*) to evaluate its effects in hematological and serum biochemical parameters. During this study, they quantified β-glucans in SOMS, obtaining an amount of 199.2 mg g^{-1}, which can be translated to 19.92 g 100 g^{-1}. Their results showed that diets containing SOMS enhanced elk physiologic condition during growth, demon-strating that even SOMS, without the need for extraction, can be used as an additive rich in bioactive polysaccharides for animal feed.

Table 3. β-glucan content in different *Pleurotus* species' fruiting bodies.

Species	β-glucans (g 100 g dry matter^{-1})
P. ostreatus	24.2
P. eryngii	15.3
P. citrinopileatus	15.5
P. pulmonarius	17.5
P. djamor	21.7

SOMS as an ingredient in aquaculture

Mushrooms and their subproducts have been aborded to be used in feed for aquaculture. Cruz-García et al. (2022) evaluated the use of mushroom meal (*Pleurotus djamor* var *roseaus*) as a partial replacement for fishmeal in diets containing 15, 20 and 25% inclusion in the diet for Nile tilapia during 60 days. Growth performances showed that all the groups of fish-fed diets with mushroom meal inclusion had significantly higher final body weights (30.1, 29.1, 27.1 g, respectively) compared with the control group (21.6 g), concluding that mushroom meal can replace fishmeal in inclusions up to 25%. For its part, Ashley-Dejo et al. (2022) used mushroom meal (*Pleurotus ostreatus*) as a replacement for maize meal in Nile tilapia diets in up to 25, 50, 75, and 100% inclusion for 56 days. Their results showed that diets with 50% substitution produced better results in feed conversion ratio, final weight, and specific growth rate, concluding that it can be used as a replacement for maize meal, which could be a scarce raw material in some seasons.

If *Pleurotus* mushroom meal has been proposed as a protein ingredient for Nile tilapia diets, animal feed ingredients should not compete with human food, so the use of by-products needs to be explored more. SOMS represents a promising raw material because, as seen in the past section, the chemical composition of substrate changes during solid fermentation, metabolizing complex lignocellulosic compounds, thus making it more digestible. Besides, mycelium enhances the spent substrate as it is rich in essential and non-essential amino acids; however, it is not able to completely degrade complex fibers, so a considerable amount is left and needs to be evaluated the amount of SOMS inclusion for tilapia feed because high fibers content decreases gastrointestinal traffic, affecting digestibility

of protein and, consequently, digestibility coefficient (De Limal et al. 2012).

Tilapias are mainly herbivorous and can utilize carbohydrates with complex sugars than disaccharides and monosaccharides; however, the quality of carbohydrates for tilapia is defined by their fiber content as increasing concentrations may depress stomach emptying, carbohydrate digestion and absorption, fish growth, and feed conversion efficiency (El-Sayed 2020). Several studies realized by Lanna et al. (2004) compared the effects of apparent digestibility and gastrointestinal transit in Nile tilapia with different crude fiber levels, namely 6, 9, and 12%, concluding that it is possible to use levels up to 9% of crude fiber in a diet for Nile tilapia alevin, and its excess may decrease weight gain and protein efficiency rate, thus resulting in lower production rates. In Table 1, the highest crude fiber is 29.7%, so in theory, as an ingredient for Nile tilapia alevins, it can be included in up to 30% if it is the only fibrous ingredient in the diet to avoid adverse effects.

SOMS has already been proposed as a safe ingredient for Nile tilapia. Faiza et al. (2020) incorporated rice straw spent by *Pleurotus* as an economical energy source replacing fishmeal, demonstrating that up to 20% can be added without consequences in growth performance, protein utilization, and economic efficiency. Thus, SOMS can also be enriched with a second fermentation, which enhances its composition, making it more digestible and resulting in better growth performance and weight gain. Katya et al. (2014) evaluated the use of fermented mushroom by-products in the Amur catfish (*Silurus asotus*) diet; the second fermentation consisted of the use of *Lactobacillus* and yeast, obtaining a final composition of 42% crude protein, 0.3% crude lipid, and 8.2% crude ash on a dry-matter basis. Even though the fermentation process was not reported, it is interesting how it is possible to convert a non-protein source, such as a lignocellulosic substrate, into a high-quality protein source through a second biotechnological process with the use of unicellular protein from yeast and bacteria.

Some other studies using SOMS as an ingredient for aquafeed have been used in catfish (*Clarias gariepinus*) (Zakaria et al. 2021), where its use resulted in safety and an enhanced survival rate of 30% during the feeding trial. Fish showed major digestibility; no differences were observed in growth performance than in the control group. However, more profound studies were not performed to understand the higher survival rate. As described in this article, SOMS contains antioxidants and polysaccharides with bioactive properties that may be responsible for those qualities, but further evaluations are necessary to elucidate the mechanism of a specific compound with this effect, turning SOMS into a potential ingredient that can be incorporated in fish feed with economic impacts such as tilapia, carp, or catfish.

This section shows that SOMS can be considered a protein source depending on the biotechnological process used to enhance its content (with mycelium as the source). Although *Pleurotus* degrades substrate fibers to grow and develop, much fiber remains in the substrate. More research should be conducted to evaluate the quantity of SOMS that can be used as a replacement for fish meal and its effect on growth. However, the bioactive properties of SOMS can be exploited as small amounts of those compounds are required, and concerns about fiber composition can be reduced.

SOMS as immunostimulants in Nile tilapia

β-glucans, in aquaculture, are one of the most used and studied compounds recognized because of their immunostimulatory activity (Leyva-López et al. 2020). They enhance and modulate the natural responses of the organisms against diseases caused by external pathogens (Manoppo et al. 2015). This effect has been in the scope of recent studies mainly because it is an alternative to avoid antibiotics, which may cause long-term problems such as loss in the environmental and gut microbiota population and even the development of resistant bacteria (Banerjee & Ray 2017, Makled et al. 2017). Besides, the reduction in the use of antibiotics may be beneficial for producers whose costs greatly enhance their uses.

The main effects associated with immunostimulants in fish can be evaluated with different biomarkers, and according to Leyva-López et al. (2020), they can be listed in four groups:

1. Increase in enzymatic activity such as lysozyme. This enzyme can deploy microbicidal activity affecting Gram-positive bacteria's cell walls by hydrolyzing peptidoglycans (Syngai & Ahmed 2019).

2. Increase in respiratory burst. Nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase is triggered by neutrophils and macrophages responding to the presence of pathogens. Consequently, superoxide anions are generated (O^{2-}) and can be measured by the nitroblue tetrazolium (NBT) reduction method to serve as an indicator of the phagocytic capacity of immune system cells (Thomas 2017).

3. Increase in blood cells (white and red cells). An elevation in erythrocyte (red cell) count suggests the presence of a substance causing collateral damage within the body. Conversely, a rise in leukocyte (white cell) count signifies an intensified immune system response to a potential infectious agent. Other blood cell indicators that can be considered are neutrophil count, hematocrit, hemoglobin level, and others (Vallejos-Vidal et al. 2016).

4. Other immunological parameters. Soluble proteins, enzymes, and receptors act in the signaling process, opsonization of pathogenic microbes, phagocytosis, and microbial destruction (Clos & Mold 2008). Immunoglobulins (Ig) and protein levels are also evaluated as immunological parameters (Vallejos-Vidal et al. 2016). Melano-macrophage centers (MMCs), pigmented phagocytic cells (melanin) that act as a rapid response to the presence of an infection; cytokine levels such as interleukin-1 (IL-1), IL-6, interferon-gamma (IFN-γ) are also considered markers of the immune response in fish (Agius & Roberts 2003).

SOMS is mainly composed of mycelium, which, in turn, is composed of insoluble β-glucan in the β-1,3/β-1,6 structure, which had a more substantial biological activity than those obtained from cereals with β-1,3/β-1,4 chains due to its structural differences (Hu et al. 2022), the former obtained from *Pleurotus* species are called pleuran (Faugeron-Girard et al. 2020). β-glucan can be administrated in different routes in fish: oral administration, intraperitoneal injection, and immersion; however, depending on the administration route, variable effects can be expected in fish, and not all of them are viable for use. Intraperitoneal administration needs technical knowledge, is laborious and stressful for the organism, and needs to be done during the fish's first stages, as well as is time-consuming and expensive (Ching et al. 2021). Immersion is not a popular method since β-glucans β-1,3/ β-1,6 are insoluble, and fish cannot absorb them correctly, and it is more suitable for fish during their first life stages (Selvaraj et al. 2005). Oral administration is the most popular method since it is non-invasive and cost-effective as it is administrated in the food, it has a long-lasting effect, no technical knowledge is required, and it is a very efficient way for mass delivery regardless of fish size (Ching et al. 2021).

The absorption of β-glucans occurs in the intestine (Fig. 2); however, only fractions <1 μm can pass through the intestine wall into the blood circulation and reach targeted organs (Selvaraj et al. 2005, Sandvik et al. 2007). The non-digested fractions in the intestine are recognized by specific receptors located in the plasma membrane named pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), C-type lectin receptors (CLRs), and complement receptor type 3 (CR3), which can recognize β-glucan molecules and initiate the downstream signaling to express immune cells (Goodridge et al. 2011, Kiron et al. 2016, Kanjan et al. 2017).

In freshwater fish, particularly Nile tilapia, the use of β-glucans has been widely explored. Some reports indicate that their consumption improves productive performance and hepatosomatic indices and increases the content of red blood cells, white blood cells, lymphocytes, monocytes, phagocytic activity, superoxide dismutase, and catalase (Şahan & Duman 2010, Barros et al. 2014, Ahmed et al. 2017). The main concentrations in tilapia feed typically range but are not limited to 0.2 to 5 g kg^{-1}, with varying effects (Ching et al. 2021). However, it is essential to note that low doses (0.1-0.3%) induce an adequate immune response, while overdoses (1.0-2.0%) can cause immunosuppression in these organisms (Raa 1996, 2000, Sakai 1999, Cook et al. 2001).

Couso et al. (2003) critically observed feeding duration with glucans. They noted that protection against pasteurellosis caused by *Photobacterium damselae* highly depended on the duration and dose. In their experiment, fish-fed glucans for two weeks showed no protection against the disease at higher inclusion levels. Only the lowest level (1.0 g kg^{-1}) provided measurable protection against the disease, while the highest level (10 g kg^{-1}) made the fish more susceptible to the pathogen. When fed for one week, glucan treatments effectively reduced disease incidence. They displayed a typical dose-response effect, which suggests that when glucans are administered through feed, in addition to considering the concentration used, they must be provided for a sufficient duration to trigger an immunostimulatory effect. This required duration may vary among different species. Based on this assumption, if SOMS were to be used as a β-glucan source to act as an immunostimulant, and considering the quantification by Park et al. (2012) at 199.2 mg g^{-1}, approximately 5 g of SOMS per 100 g of diet would be required to achieve a β-glucan concentration of 1 g. However, further studies are necessary to quantify β-glucan levels in different SOMS types and assess their potential immunomodulatory effects in fish. The use of mushrooms and their by-products as immunostimulants has already been proposed for Nile tilapia. Van-Doan et al. (2017) worked with Nile tilapia in a feeding trial for eight weeks by incorporating spent substrate from *Cordyceps*

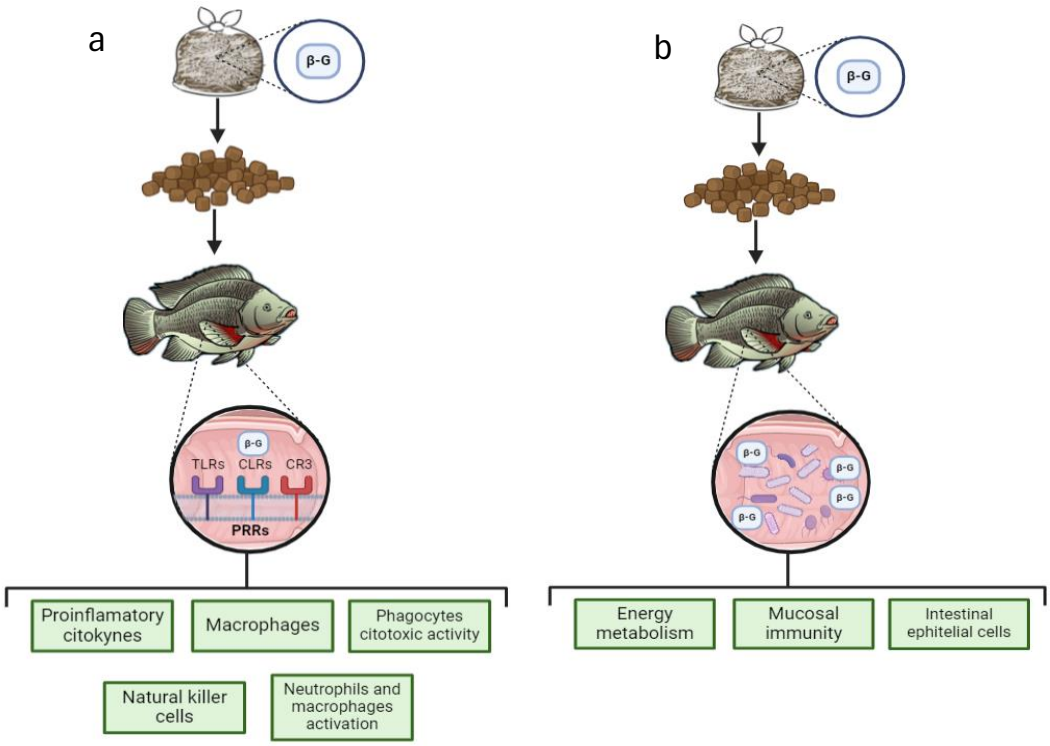

Figure 2. β-glucan (β-G) from spent oyster mushroom substrate can be administered through diet. Once it reaches the intestine, a) recognition of β-glucan occurs by specific receptors located in intestine cells' plasma membrane**,** thus promoting downstream signaling to express immune cells, and b) microbiota modulation can occur by stimulating bacteria able to take advantage of β-glucan to grow and develop thus producing enzymes and enhancing mucosal well-being.

militaris and in combination with *Lactobacillus plantarum* to evaluate immunological and growth parameters, finding that experimental diets significantly increased skin mucus production, lysozyme, and peroxidase activities thus, stimulating fish immune system due to spent mushroom substrate and *L. plantarum* synergy. Authors associated the enhanced effect with molecules related to *Cordyceps*, such as cordycepin and adenosine, which have essential biological functions since the spent mushroom substrate is fermented by specific intestinal bacteria whose fermentation products can improve immune function and growth performance. Nonetheless, the mechanisms behind the immunomodulatory effect in fish still need to be clarified.

Chirapongsatonkul et al. (2019) tested the use of spent mushroom substrate of split gill mushroom (*Schizophyllum commune*) crude glucan extract and compared its effects in Nile tilapia against glucans derived from yeasts in an intraperitoneal administration. The crude glucan from spent mushroom substrate enhanced immunological parameters (lysozyme activity, bactericidal activity, mucosal enzymes) and expression of genes related to immune cells just as well as glucans from yeast, which makes extracts from spent mushroom substrate a cheap and essential source of molecules with immunostimulatory effect.

SOMS has not been explored yet as a source of β-glucan for fish diets; other sources, such as stalk wastes, have been considered (Ching et al. 2022). In this study, an ethanolic extract was obtained and incorporated into the diet of red hybrid tilapia and challenged with pathogen-associated molecular patterns. As a result, the extract modulated cellular and humoral immune responses and immune-related genes and even improved fish growth performance.

Well, spent mushroom substrate from sources other than oyster mushrooms has already been explored in Nile tilapia. There is still a huge opportunity to explore SOMS as a specific source of β-glucan and compare its effect with a common source, such as yeast, to see if they have similar immunostimulant effects. Besides, it

is necessary to evaluate the use of SOMS as a whole matrix and not just its extracts to avoid producing more residues.

Intestinal microbiota modulation of fish

Fish natural microbiomes in the intestine comprise a complex of dynamic communities. They are essential in maintaining intestinal activities, such as nutrient absorption and metabolisms that help the organism reach homeostasis (Llewellyn et al. 2014, Tarnecki et al. 2017). Thus, disruptions in these communities can lead to disease.

Recent studies have started to focus on how certain ingredients have an effect that may either increase or decrease gut microbiota richness to improve fish growth performance. β-glucan administration in diets can increase gut microbiota richness and diversity (Jung-Schroers et al. 2016, Miest et al. 2016). Beneficial bacteria can aid in energy metabolism (Fig. 2) and the development of epithelial cells, which are essential immunocompetent cells. These cells are responsible for enhancing the physical barrier of the intestine by producing innate humoral defense peptides and proinflammatory cytokines (Komatsu et al. 2009, Gomez et al. 2013, Dawood et al. 2020). Souza et al. (2020) evaluated the impact of β-glucan inclusion in Nile tilapia during a hypoxia trial on changes in immune system parameters and gut microbiota. As a result, the authors found an abundant genus that can metabolize different carbohydrate sources through various metabolic pathways, such as *Rombutsia*, an abundant genus identified in tilapia, and the greater abundance was observed for the members of the *Vibrionaceae* family. However, the authors were not able to classify them. They discuss that certain members of this family may exhibit pathogenic, symbiotic, or probiotic characteristics depending on the genus and/or strain of each species, so further investigations need to be done to evaluate which certain families are stimulated with the addition of β-glucan and its relationship with fish well-being. Xu et al. (2020) evaluated the effect of β-glucan supplementation in Nile tilapia under hypersaline stress; in their results, they found that the addition of β-glucan increased significantly beneficial genera such as *Lactobacillus, Phycicoccus,* and *Rikenellaceae* in fish gut and were able to improve fish health under saline stress.

Nevertheless, further studies need to be done to understand how β-glucan, from different sources such as SOMS, can modulate gut microbiota and its relation in fish immune responses under different stress conditions as they may serve as prebiotics. In our work group, we explored the effects of prebiotics such as agavin (Flores-Méndez et al. 2024) in microbiota modulation of Nile tilapia and its possible correlation between the stimulation of certain filum and growth parameters. In this recent publication, they found that agavin can stimulate the growth of potentially beneficial bacteria such as Sphingomonadaceae, Oxalobacteriaceae, and Chitinophagaceae, reducing the abundance of pathogenic families such as Vibrionaceae and Aeromonadaceae in tilapias under high-density cultivation.

SOMS is a fibrous material rich in β-glucans and serves as a pre-digested raw material, so its use as a whole matrix could be directed to herbivorous fish and serve as a prebiotic. Its potential to positively influence the intestinal microbiota of Nile tilapia presents an exciting area for evaluation. By investigating its effects on microbiota health, SOMS could be proposed as an affordable and sustainable source of β-glucans for aquaculture applications.

CONCLUSION

Mushroom by-products, particularly *Pleurotus* mushrooms, hold immense potential as valuable raw materials for aquaculture, especially for including Nile tilapia feed as an additive with antioxidant and immunostimulant activity. The ability of *Pleurotus* mushrooms to degrade lignocellulosic material enhances its digestibility and chemical composition, with notable improvements in protein content. Additionally, SOMS is rich in bioactive compounds like antioxidants and polysaccharides, which, when orally administered, can promote overall organism well-being as they can act as immunostimulants. These by-products represent a cost-effective ingredient that could reduce ecological management burdens, given their lack of commercial value. Furthermore, exploring their impact on intestinal microbiota presents a significant opportunity, as research suggests its profound influence on fish health modulation.

Credit author contribution

J.I. García-Aguirre: conceptualization, writing-original draft, review and editing; C. Hernández: validation, supervision and editing; M. Esqueda: final validation, review and editing. All authors have read and accepted the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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