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Prebiotic effects of dietary xylooligosaccharides on fish gut microbiota, growth, and immunological parameters – a review

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Highlights

- Xylooligosaccharides (XOSs) can have promising prebiotic effects on fish performance.
- XOSs can modulate the gut microbiota and increases the production of SCFAs in fish.
- XOSs can strengthen the innate immune system and disease resistance of fish.
- The effects of XOSs depend on the structure, source, dosage, and fish species.

Abstract

Xylooligosaccharides (XOSs) are increasingly being explored as prebiotics in fish diets; however, their effects and modes of action have not been well evaluated. Reports have shown that dietary XOS has the potential to improve the proliferation of beneficial gut microbes, and their metabolites, and enhance disease resistance in several fish species. In contrast, other studies report no substantial changes in immune and growth parameters compared to control groups. Like all prebiotics, the mode of action of XOS is based on their selective stimulation of beneficial gut microbiota, which will outcompete and prevent pathogen proliferation in the gut, and produce metabolites that modulate host immune responses. The reports of improved growth performance of XOS fed fish may be due improved intestinal microbiome, enhanced glycolysis activity and elevated gastrointestinal enzymatic activities. Dietary XOSs have different effects on fish performance depending on the fish species and the structure of XOSs (degree of XOS polymerization and substitution). Nevertheless, further research is essential to determine the optimal dosage, degree of polymerization, and substitution levels required to improve each fish species' gut health and growth performance. This review highlights the prebiotic effects of XOSs, their mechanism of action, and knowledge gaps.

Key words: prebiotics, xylooligosaccharides, fish gut microbiota, fish immunity, fish growth performance

Fish farming is the world's fastest-growing agri-business sector, contributing to global food security, poverty alleviation, income generation, and employment (Subasinghe et al., 2009; Kawarazuka and Béné, 2010; FAO, 2021). Fish provide a low-cost source of protein to many low- and middle-income countries. Farmed fish production in 2020 was 57.5 million tons (FAO, 2021). Despite the increased fish productivity in recent years, fishery products have a global scarcity. In addition, fish cultured in intensive systems may be more susceptible to infectious diseases if the systems are not adequately maintained because of poor water quality, increased stress, and transboundary pathogens (owing to increased trade) (Subasinghe, 2009).

Infectious diseases have caused significant economic losses in intensive aquaculture (Qi et al., 2009; Subasinghe, 2009; Dawood et al., 2018). Some bacterial infections can be prevented and controlled by adding antibiotics and chemotherapeutics to aquafeeds. However, the use of antibiotics as feed additives in aquaculture is banned in many countries. The primary

reasons for prohibiting antibiotics as feed additives are the global expansion of antimicrobial-resistant bacteria, weakened immunity, antimicrobial residues in fish tissues (human health safety concerns), and adverse environmental effects (Qi et al., 2009; Santos and Ramos, 2018). This has resulted in the development of safer, more ecologically acceptable, cost-effective natural ingredients and non-antibiotic supplemental alternative feed additives such as phytobiotics, prebiotics, and probiotics for managing and controlling microbial infections in aquaculture (Merrifield et al., 2010a; Merrifield et al., 2010b; Song et al., 2014; Guardiola et al., 2016; Wang et al., 2017; El-Saadony et al., 2021; Abdel-Latif et al., 2022; Abd-Elaziz et al., 2023; Abdel-Latif et al., 2023).

It is now commonly acknowledged that the function and composition of the gut microbiota contribute to the preservation of host gut health and, as a result, systemic host health. Ecological alternatives to antibiotics such as feed additives can influence the function and composition of fish gut microbes. Understanding how these environmental feed alternatives affect the gut microbiota is one of the most significant advances in using functional feeds in aquaculture. Disturbed microbiota has been linked to several diseases in fish. Researchers have been working on dietary feed additives to rectify or restore these disorders.

Prebiotics are primarily non-digestible carbohydrates such as inulin, xylooligosaccharide (XOS), fructooligosaccharides (FOS), galactooligosaccharide (GOS), transgalactooligosaccharides, lactulose, isomaltooligosaccharides, lactosucrose, soybean oligosaccharides, glucooligosaccharides. and mannanoligosaccharide (MOS), which are incorporated into aqua-feeds and are resistant to host gastric acids or hydrolytic digestive enzymes and are selectively used by beneficial gut bacteria, which normally occupy the intestine (Roberfroid, 2007; Gibson et al., 2010). Prebiotics have been documented to improve growth performance, immune system, beneficial gut microbiota, and availability of critical vitamins and proteins in fish (Mussatto and Mancilha, 2007; RingØ et al., 2010; Hoseinifar et al., 2016b; Guerreiro et al., 2018a). Generally, prebiotics promotes the proliferation of beneficial gut microbiota, which limits the spread of pathogenic microorganisms by limiting adhesion sites, modifying gut pH, producing antimicrobials (e.g., bacteriocins), lowering virulence, and activating host immunity (Mussatto and Mancilha, 2007; RingØ et al., 2010; Hoseinifar et al., 2016b; Guerreiro et al., 2018a). Furthermore, prebiotics improves glucose absorption, trace element bioavailability (vitamins and minerals), and short-chain fatty acid (SCFAs) production (Bongers and van den Heuvel, 2003; Burr et al., 2005; Mussatto and Mancilha, 2007). In addition, prebiotics have been shown to increase the survival rates of numerous fish species against pathogenic microorganisms (Hoseinifar et al., 2016b; Guerreiro et al., 2018a; Torrecillas et al., 2018).

A number of prebiotics have been used in aquaculture to boost growth performance, immunological response, and disease resistance among certain fish species (Grisdale-Helland et al., 2008; Burr et al., 2010; Merrifield et al., 2010b; Hoseinifar et al., 2013). XOSs have recently attracted the attention of researchers for potential use in aquaculture (Guerreiro et al., 2015b; Van Doan et al., 2018; Van Doan et al., 2020; Morshedi et al., 2018). XOSs are xylose-linked (-(1)4)-linkages) oligomers formed by steam, chemical, and enzymatic hydrolysis of xylan-containing lignocellulosic biomass (Aachary and Prapulla, 2011). In general, the technique of manufacture and purification to be utilised is decided based on the use and amount of XOSs (Aachary and Prapulla, 2011). XOSs have different structures depending on the xylan

source, monomeric units, degree of polymerization (DP), degree of substitution (DS), type of linkage, and production method (Aachary and Prapulla, 2011). Compared to other prebiotics such as FOS, inulin, GOS, and MOS, relatively few studies have evaluated the prebiotic effects of XOSs in fish. This review provides an overview of the prebiotic effects of XOSs and their mode of action in fish, as well as identify current knowledge gaps.

The effect of XOS source, manufacturing process, and structure

Sources of XOSs

Compared to other oligosaccharides, XOSs have lower viscosity, which can minimise water activity and increase water retention (Peng et al., 2011). Since XOSs are soluble in water, they are strongly recommended to be pelleted before being fed to fish. High-quality fish feed pellets are less likely to swell in water and have more water resistance, which keeps them from disintegration. As a result, the feed better retains its constituents until it is consumed. Different structures of XOSs have been observed depending on the source of xylan used in the synthesis, monomeric units, degree of polymerization, nature of the linkages, and their combination with side groups (Aachary and Prapulla, 2011). Adding side groups (arabinose residues, acetyl groups, or glucuronic acid) results in different XOSs profiles with other biological characteristics and properties (Aachary and Prapulla, 2011). Various xylan-containing lignocellulosic biomasses such as corncobs, bamboo shoots, fruits, citrus peels, vegetables, sugarcane bagasse, hardwood raw materials, softwood raw materials, barley husks, barley spent grain, rice hulls, rice husks, rye, meranti wood sawdust, cassava, brewery spent grains, almond hulls, oat husks, beech wood, birch wood, wheat straw, corn husks, cotton stalks, and corn fibres have been used to produce XOSs (Aachary and Prapulla, 2011). XOSs made from corn cobs using *Aspergillus* xylanase in Nile tilapia showed prebiotic effects (Van Doan et al., 2018; Van Doan et al., 2020). Morshedi et al. (2018) used XOS from corncob and found no significant prebiotic effects compared to the control group. Azerodo et al. (2017) discovered that XOS from corncob increased lysozyme activity but had no effects on total immunoglobulins, bactericidal activity, or complement content in experimental trials. Guerreiro et al. (2015b) discovered that XOS from corncob increased weight gain, catalase activity, glutathione peroxidase activity, protein efficiency rate, glycolytic activity, feed conversion rate, and total superoxide dismutase, while it decreased lysozyme activity. Even though some studies did not specify the method of production or the lignocellulosic biomass used, it is likely that the respective manufacturers have not used identical production and processing methods, or identical XOS sources. Therefore, the type of biomass and production technique should be carefully considered since these are likely to be factors affecting efficacy.

The structure of XOSs

Different XOS structures (DP and degree of substitution) affect the function and structure of gut microbiota differently. Therefore, the ability of beneficial gut microbes to utilize XOSs may explain the differences in the modulation of the gut microbiota of fish. *In vitro* tests have shown that different probiotics can degrade XOS with varied efficiencies. For example, *Bifidobacterium adolescentis* ferments XOS from rice husks faster than *B. longum*, *B breve*, and *B. infantis* (Gullón et al., 2008). After 24 h, *B. adolescentis* consumed 77% of XOS,

with xylotriose (90%) being the most utilized, followed by xylobiose (84%), xylotetraose (83%), and xylopentaose (71%) (Gullón et al., 2008).

Based on the *in vitro* utilization of XOSs by probiotics/beneficial bacteria, it is likely that most of the prebiotic benefits of dietary XOSs are generated from components with a low average DP. However, some probiotics/beneficial gut bacteria might favour xylotriose over xylopentaose, xylohexaose, and mixed XOSs. Further studies are needed to determine how the DP of XOSs influences the gut microbiota of fish. The degree of substitution of XOSs by beneficial gut microbes can be assessed using bacterial fermentation and proliferation rates. XOSs are often substituted with various substituents such as arabinose, phenolic compounds, glucuronic acids, and acetyl groups, and the more XOSs are substituted, the fewer beneficial gut microbes are present (Aachary and Prapulla, 2011). This implies that bacteria prefer unsubstituted XOSs over substituted XOSs. It has been shown that unsubstituted and arabinose-substituted XOSs are fermented faster than XOSs substituted with acetyl or glucuronic acid (Aachary and Prapulla, 2011). In addition, the side chain groups of ferulic acid play a role in the fermentation of arabinose-XOS. The more ferulic acid groups present, the more difficult it is for arabinose-substituted XOS to be degraded by microbial enzymes (Aachary and Prapulla, 2011).

In vitro fermentation of XOSs by *Bifidobacterium* species and other lactic acid producing bacteria (LAB) revealed acetate as the predominant short-chain fatty acid (SCFA) produced, followed by propionate and butyrate. The amount of SCFAs in the fish diets varies depending on the dose, fish species, and XOS structure (Geraylou et al., 2012; Geraylou et al., 2013a; Geraylou et al., 2013b; Sun et al., 2021). According to Geraylou et al. (2012; 2013a; 2013b), the major SCFAs produced in the hindgut of Siberian sturgeons were acetate, propionate, and butyrate, with acetate being the most abundant. The acetate concentration in the hindgut of fish varied depending on the XOSs DP, and the degree of substitution with AXOS-32-0.30 significantly increased the amount of acetate compared to AXOS-3-0.25. In the Siberian sturgeon, butyrate levels were higher in fish fed on AXOS-32-0.30 than in fish fed on AXOS-3-0.25 and control diets (Geraylou et al., 2012). Propionate was produced at low levels in all treatments, with no discernible differences in propionate concentrations (Geraylou et al., 2012). In addition, total SCFAs were significantly higher in AXOS-32-0.30-fed fish than in AXOS-3-0.25-fed and control fish (Geraylou et al., 2012). Sun et al. (2021) found that acetate and propionate levels were significantly increased in the intestines of grass carp fed on 60 mg XOS/kg diet. Although butyrate levels were low in all XOS-fed fish groups, butyrate concentrations in the intestines of fish provided with 40-100 mg XOS/kg diet were significantly higher than those in the control group (Sun et al., 2021).

The presence of butyrate in these *in vivo* fish studies indicated that other beneficial gut microorganisms could ferment XOSs, producing SCFAs that are not usually made by *Bifidobacterium* and *Lactobacillus* species. These beneficial butyric acid-producing bacteria can ferment acetate and lactate to produce butyric acid. This phenomenon has been linked to the cross-feeding mechanism of intestinal bacteria. Cross-feeding has been well studied in humans (Belenguer et al., 2006) and broilers (De Maesschalck et al., 2015), with *Bifidobacterium* and *Lactobacillus* species producing lactate and acetate, which are subsequently used by beneficial butyrate-producing bacteria such as *Clostridium butyricum* to produce butyrate (Belenguer et al., 2006). Therefore, studying cross-feeding mechanisms in

fish is crucial for understanding the interactions between healthy gut microbiome constituents. Exploring new probiotics that degrade XOSs and produce beneficial SCFAs in the host is also important. For example, Hoeseinifar et al. (2015b) discovered that *in vitro* fermentation of XOSs by *Pediococcus acidilactici* produces propionic and butyric acids. Ultimately, this could serve as a basis for developing methods to find and select the best synbiotic interventions to create a variety of beneficial metabolites, such as SCFAs. The fermentability and prebiotic effects of XOSs on fish vary depending on their properties (Gullón et al., 2008; Rurangwa et al., 2009; Aachary and Prapulla, 2011; Hoseinifar et al., 2017; Petrova and Petrov, 2017).

Utilizing XOSs by beneficial gut microbes requires a comprehensive and efficient repertoire of xylanolytic metabolic enzymes and cellular transport mechanisms/systems. However, XOSs enter the cells of beneficial gut microbes via specific transporters (ATP-binding cassette transporter system, ABC) and are degraded by cell-associated or intracellular enzymes such as β-1,4-xylosidases, β-arabinosidases, β-glucuronidase, and acetyl-xylan esterase (Aachary and Prapulla, 2011; Petrova and Petrov, 2017). The efficacy of the xylanolytic enzyme systems of *Bifidobacterium* species and LAB determines their ability to digest XOSs. Petrova et al. (2017) found that XOS metabolism is better defined in *Bifidobacterium* species than in other probiotic bacteria and yeasts. However, it should be noted that *Bifidobacterium* species are not a common or abundant component of the gut microbiota of fish species (Romero et al., 2014; Ringø et al., 2016; Luan et al., 2023). In a recent study by Iliev et al. (2020), three LAB strains (*L. brevis*, *L. plantarum*, and *L. sakei*) were tested for their ability to utilise and grow on XOSs; it was demonstrated that these strains utilised shorter XOSs first and formed metabolites of characteristic mixed-acid fermentation. Furthermore, the discovery of intracellular β-D-xylosidase in *L. brevis*, *L. plantarum*, and *L. sakei* suggests that XOSs may be first imported into the cell by oligosaccharide transporters and then degraded to xylose (Iliev et al., 2020). Further research is required to determine the XOSs consumption pathways of other LAB probiotics, which will aid in the formulation of synbiotic products.

Different production methods of XOSs

XOSs are composed of xylose oligomers linked by β-(1,4)-linkages and is produced from xylan-containing lignocellulosic biomass via autohydrolysis (high-temperature steaming), chemical hydrolysis, and enzymatic hydrolysis (Aachary and Prapulla, 2011). There are several dynamic techniques for XOSs generation and purification, and their choice depends on the amount and use of the XOSs (Aachary and Prapulla, 2011). A combination of these approaches produces XOSs quickly and effectively. Xylan-containing biomass is heated after treatment with an alkali such as potassium hydroxide or sodium hydroxide to produce xylan, which is then hydrolysed by xylanase enzymes to produce XOSs (Akpinar et al., 2007; Aachary and Prapulla, 2011). Combining chemical, thermal, and enzymatic hydrolysis may make XOSs with fewer undesirable by-products and monosaccharide sugars (Aachary and Prapulla, 2011). Different enzymes hydrolyse agricultural wastes in different ways, resulting in various XOSs profiles, whereas *in vitro* utilization of XOSs by various probiotics reveals different growth patterns of the resulting XOS (Gufe et al., 2021).

Prebiotic effects of XOS on fish parameters

Effects on intestinal microbiota

The host and gut bacteria constantly interact and these interactions regulate numerous biological processes (physiological responses and activities) in humans, animals, and fish (Rawls et al., 2007; Nayak, 2010; Sekirov et al., 2010; Sullam et al., 2012). Healthy gut microbiota prevent pathogenic bacteria from spreading and invading the gut (outcompeting pathogens for adhesion sites and resources and producing metabolites that inhibit pathogen growth), improve the growth performance and innate immunity, and increase the disease resistance in fish (Dimitroglou et al., 2011). Therefore, the homeostasis of fish gut microbiota is critical for fish health and growth. Previously, microbial communities in the fish gut have been regulated using microbial load reduction techniques such as antibiotics to feed. However, the adverse effects of antibiotics, such as the inhibition of beneficial gut microorganisms and the development and spread of antibiotic resistance, have led to the use of eco-friendly alternative feed additives such as probiotics, prebiotics, and herbal preparations. These feed additives can help to limit or reduce the use of antibiotics (Qi et al., 2009; Santos and Ramos, 2018). Prebiotics, such as XOSs, have modulated the gut microbiota in several fish species.

As shown in Table 1, in some studies, XOSs selectively promotes the proliferation of autochthonous/resident beneficial gut microbes (with higher relative abundance in the XOSs-fed fish groups than in the control groups i.e., fish-provided diets without XOSs enrichment) and reduce the relative abundance of pathogenic bacteria or adherent heterotrophic bacteria (Hoseinifar et al., 2016a; Guerreiro et al., 2018a; Poolsawat et al., 2021; Sun et al., 2021). According to 16S rRNA gene sequencing libraries (Geraylou et al., 2012; Geraylou et al., 2013b), the relative abundance of Firmicutes increased in juvenile Siberian sturgeon (*Acipenser baerii*) fish fed on XOSs diets, whereas the abundance of Fusobacteria decreased. However, the relative abundance of Proteobacteria was not significantly affected, compared with the control group. The abundances of *Rhodobacter* spp., *Aeromonas* spp., *Escherichia coli*, *Citrobacter freundii*, and *Cetobacterium somerae* were statistically significantly reduced at the species level. In contrast, *Clostridium colicanis*, *C. beijerinckii*, *Lactococcus raffinalactis*, *L. lactis*, *C. baratii*, *Candidatus arthromitus*, *Eubacterium budayi*, and *L. aviaries* were significantly increased (Geraylou et al., 2012; Geraylou et al., 2013b). The relative abundance of *L. lactis* was the same in both XOSs-fed and control fish groups (Geraylou et al., 2012; Geraylou et al., 2013b). When comparing arabinoxylan-oligosaccharides (AXOS) of different DP (AXOS-32-0.30 vs AXOS-3-0.25), they observed that *C. colicanis*, *L. aviaries*, *L. raffinalactis*, *C. baratii*, *E. budayi*, and *L. lactis* were only present in the fish fed on AXOS-32-0.30. Poolsawat et al. (2021) found that the microbial community diversity index (Shannon) and community richness indicators (ACE and Chao) of hybrid tilapia (*O. niloticus* × *O. aureus*) did not differ significantly between the XOSs-fed fish groups and the control group. However, the community diversity index (Simpson) was considerably higher in the XOSs-fed fish groups ($P > 0.05$) than in the control group. Guerreiro et al. (2018a) reported that dietary XOSs increased the Margalef species richness index in XOSs-fed European sea bass but Shannon's diversity index was not affected.

Few studies have investigated the effects of dietary XOS on the intestinal microbiota of different fish species. Fish gut microbiota differs depending on their location, nutrition, habitat, feeding behaviours, management, and digestive tract physiology (Rawls et al., 2006; Sullam et

al., 2012). Before administering XOSs, it is crucial to understand the unique beneficial autochthonous gut flora of the target fish species. However, variations in the reported gut microbiota might also be due to the methodology used to determine the number and diversity of gut microbiota. For example, culture-dependent techniques compared the total viable and probiotic counts between the XOSs and control-fed fish groups. Using culture-dependent techniques, Sun et al. (2021) reported that XOSs supplementation considerably reduced the number of *E. coli* and *Aeromonas* in the intestine of grass carp but significantly increased the number of *Lactobacillus* and *Bifidobacterium* spp.. However, this study only used culture-dependant enumeration technique, which will have only revealed the impacts on a minority of the microbial community. Another study on hybrid catfish (*Pangasianodon gigas* × *P. hypophthalmus*) reported that the total number of culturable viable intestinal bacteria was similar between the XOSs and control fish groups (Hahor et al., 2019). However, autochthonous LAB counts were considerably higher in fish fed the XOS diet than those fed with the control diet. Hoseinifar et al. (Hoseinifar et al., 2014; Hoseinifar et al., 2016a) reported that administering XOS to Oscar fry (*Astronotus ocellatus*) and Caspian white fish (*Rutilus frisii kutum*) significantly increased the total number of autochthonous heterotrophic gut bacteria and autochthonous LAB. Poolsawat et al. (2021) observed that feeding XOSs to Nile tilapia increased the abundance of LAB and *Bacillus* species and decreased the abundance of *E. coli*. Furthermore, the administration of 2% AXOS resulted in a significant rise in LAB abundance in the gut of Siberian sturgeon (*Acipenser baerii*) juveniles (Geraylou et al., 2013). In another study, it was reported that *Lactobacillus* species increased as the dosage increased from 0.5 to 1% dietary XOS supplementation, implying that XOS may encourage the development of certain probiotic strains or beneficial gut microbiotas (Wang et al., 2022). The disparities in findings from different studies on the effects of XOS are most likely due to differences in administration, dosages, and prebiotic fermentability, and perhaps most importantly, in the differences in the microbiota and intestinal morphologies of the respective fish populations in each study (Hoseinifar et al., 2010). Many other considerations, including the aquatic system, fish species, and developmental stages used within experimentations, can all have an impact on the baseline host microbiome and thus impacts the efficacy and outcomes of functional feed additives. In addition, the method used in studying the microbiota can influence the results, for example, culture-dependent methods can only be used to estimate culturable microorganisms. Non-culture methods, such as high-throughput sequencing of the 16S rRNA gene, have demonstrated that the prebiotic effects of XOSs may vary at the phylum, family, genus, and species levels. Based on 16S rRNA sequencing data, Guerreiro et al. (2018b) observed that there were no significant differences in microbial community richness and diversity between the XOSs supplementation groups and the control group. To better understand the modulation of fish gut microbiota by dietary XOSs, further studies using full length 16S rRNA gene sequence libraries and metagenomics is required. Although the mechanisms of dietary XOSs and prebiotic effects on fish gut microbiota have not been well investigated, XOSs, like other prebiotics, regulate the structure and function of the gut microbiota via an indirect mechanism. On the contrary, MOSs have been shown to have a direct mechanism by which they bind to pathogenic bacteria and limit their colonisation of the mucosal epithelium. Additional studies are required to elucidate any possible direct mechanisms of action of dietary XOSs on the gut microbiota of fish.

Table 1. Effects of dietary XOS on fish microbiota

Intervention	Dose	Control	Duration (Days)	Fish species	Outcome	References
XOS	1%	FWP	30	*D. labrax*	⬆Margalef species richness index ▬ Shannon's diversity index	(Guerreiro et al., 2018b)
XOS	1 %	FWP	56	*A. ocellatus*	⬆TVC, TPC	(Hoseinifar et al., 2016a)
XOS	2%	FWP	56	*A. ocellatus*	⬆TVC, TPC	(Hoseinifar et al., 2016a)
XOS	0.002 - 0.010%	FWP	60 days	*C. idella*	⬆ *Bifidobacterium* count, *Lactobacillus* count ⬇*E. coli* count, *Aeromonas* count	(Sun et al., 2021)
XOS	0.05 - 0.4%	FWP	56 days	*O. niloticus* × *O. aureus*	⬆LAB count, *Bacillus* count, community diversity (Simpson indices) ▬TVC, community richness (ACE and Chao1 estimators), community diversity (Shannon indices) ⬇*E. coli* count	(Poolsawat et al., 2021)
XOS	2.5, 5, 7.5, 10 g/kg	FWP	56 days	*Oncorhynchus mykiss*	⬆*Lactobacillus* count ⬇*E. coli* count, *Mycoplasma*	(Wang et al., 2022)

					▬TVC, *Bacillus* count, community richness (ACE and Chao index), community diversity (Shannon index and Simpson)	

AXOS-arabinoxylooligosaccharides, XOS-Xylooligosaccharides, FWP-feed without XOS supplement, TVC- Total viable counts, TPC-Total probiotic counts.

Key: ⬆-significantly increased by XOS compared to control, ⬇- significantly decreased by XOS compared to control, ▬ - no significant difference between XOS supplementation and the control

Effects of dietary XOSs on fish growth performance

Fish health status can be determined by growth parameters such as specific growth rate (SGR), weight gain (WG), feed conversion rate (FCR), conditional factors (CF), hepatosomatic index (HSI), and viscerosomatic index (VSI). Most studies in the current literature have reported statistically significant effects of dietary XOSs on WG and SGR (Table 2). The effect on WG and SGR have differed depending on XOSs structure, concentration, source, and fish species. The FCR was lower (i.e., improved) in many XOSs-fed fish groups compared to the control groups (Table 2). High WG, SGR, and low FCR are benefits that will improve fish production and profit. However, the type of XOS (DP, branched/unbranched) and dosage suitable for each application must be selected carefully considering various factors (fish species, age, size, rearing environment etc), which may affect efficacy. The XOSs dosage in aquafeeds is also a key factor to consider when administering XOSs to a particular fish, as shown in Table 2.

The mechanism by which dietary XOSs influence fish growth performance could be due to XOSs fermentation by beneficial gut microbiota, leading to additional microbial biomass or metabolites which can be used by the host. For example, SCFAs which are uptaken by epithelial cells, and oxidised as a source of energy, and help to improve glucose and lipid absorption and metabolism by the host (Guerreiro et al., 2015b; Chen et al., 2022). Dietary XOSs can effectively regulate the microbiological environment of the intestine, lower gut pH, and increase the activity of intestinal digestive enzymes, thereby improving fish digestion and absorption, decreasing FCR, and promoting improved fish growth performance (Xu et al., 2021). Dietary XOSs, according to Guerreiro et al. (2015b), reduces lipogenesis while increasing glycolytic activity. Like other prebiotics, dietary XOSs has been shown to improve glucose metabolism and the absorption of vitamins and minerals in the colon/hindgut of fish, humans and animals (Bongers and van den Heuvel, 2003; Burr et al., 2005; Mussatto and Mancilha, 2007; Guerreiro et al., 2018a; Chen et al., 2022). The intestinal epithelium utilizes SCFAs as energy sources (Guerreiro et al., 2018a). The data in Table 2 shows that XOS improved the intestinal mucosa by increasing mucosal fold width, weight and height which are

required for efficient digestion, absorption, and utilization of nutrients. The gut microbiota can regulate the gene expression of cells in the intestines, regulating barrier function and integrity. To achieve optimal efficacy, a better understanding of the resident host gut microbiomes would be useful in order to select the appropriate source and type of dietary XOSs for each fish species.

Moreover, XOSs fermentation by beneficial gut bacteria may benefit fish by providing energy from the simple soluble sugars produced and preventing the colonization of the gut by pathogenic microbes (Manning and Gibson, 2004; Vázquez et al., 2005). Systemic XOSs fermentation by *Bifidobacterium* species and LAB increases glycolysis and digestive enzyme activity (Guerreiro et al., 2016; Morshedi et al., 2018), resulting in improved growth performance and body composition of fish. However, adding XOSs to the diet of *S. hasta* did not affect intestinal lipase and amylase activities (Morshedi et al., 2018b). The contradictory results obtained in different studies may be attributed to the form and dosage of XOSs, the feeding period, culture conditions, fish species, sample collection methods, and techniques used to assess the activity of the various enzymes (Hoseinifar et al., 2014). Dietary XOSs have been reported to increase fish muscle protein, dry matter, and ash content while lowering fish meat's glucose and cholesterol levels (Abasubong et al., 2018a; Chen et al., 2022).

Table 2. Effects of dietary XOSs on growth parameters of fish

Intervention	Dose	Control	Duration (Days)	Fish species	Outcome	References
XOS		FWP	49	*D. labrax*	⬆WG, FCR, PER	(Guerreiro et al., 2015b)
Corncob derived-XOS produced using crude xylanase from *A. niger*	1%	FWP	84	*O. niloticus*	⬆WG, SGR ⬇FCR	(Van Doan et al., 2020)
			56		⬆WG, SGR ⬇FCR	
			28		⬆WG, SGR ⬇FCR	
Corncob derived-Corncob derived-XOS produced using crude xylanase from *A. niger*	0.5%	FWP	56	*O. niloticus*	⬆WG, SGR, ⬇FCR	(Van Doan et al., 2018)
			28		⬆WG, SGR ⬇FCR	
Corncob derived-XOS produced using crude xylanase from *A. niger*	1%	FWP	56	*O. niloticus*	⬆WG, SGR ⬇FCR	(Van Doan et al., 2018)
			28		⬆WG, SGR ⬇FCR	
	2%	FWP	56		⬆WG, SGR	

Corncob derived-XOS produced using crude xylanase from *A. niger*				*O. niloticus*	⬇FCR	(Van Doan et al., 2018)
			28		⬆WG, SGR ⬇FCR	
XOS	0.5 %	FWP	56	*M. amblycephala*	⬆FCR ⬇WG, PER, SGR.	(Abasubong et al., 2018a; Abasubong et al., 2018b; Abasubong et al., 2019)
XOS	1.5 %	FWP	56	*M. amblycephala*	WG, PER, SGR ⬇FCR	(Abasubong et al., 2018a; Abasubong et al., 2018b; Abasubong et al., 2019)
XOS	2.3 %	FWP	56	*M. amblycephala*	⬆FCR ⬇PER, WG, SGR	(Abasubong et al., 2018a; Abasubong et al., 2018b; Abasubong et al., 2019)
XOS	3 %	FWP	56	*M. amblycephala*	⬆FCR ⬇PER, WG, SGR	(Abasubong et al., 2018a; Abasubong et al., 2018b; Abasubong et al., 2019)
XOS	0.5%	FWP	56	*Sparidentex hasta*	▬WG, FCR, SGR, LA, LiA, HF, AA, BC ⬇Pase	(Morshedi et al., 2018)
XOS	1%	FWP	56	*S. hasta*	▬WG, FCR, SGR, LiA, HF, AA, BC ⬇Pase	(Morshedi et al., 2018)
Corncob derived-XOS produced using Endoxylanase (PoXyn2) from *Penicillium*	0.5%	FWP	84	*D. labrax*	⬆WG, FCR, SGR, PER,	(Abdelmalek et al., 2015)
			56	*D. labrax*	⬆WG, SGR ▬FCR, PER	(Abdelmalek et al., 2015)

occitanis expressed in *Pichia pastoris*						
Corncob derived-XOS produced using Endoxylanase (PoXyn2) from *P. occitanis* expressed in *Pichia pastoris*	1%	FWP	84	*D. labrax*	⬆WG, SGR ▬FCR, PER	(Abdelmalek et al., 2015)
			56	*D. labrax*	⬆WG, FCR, SGR, PER ▬FCR, PER	(Abdelmalek et al., 2015)
XOS		FWP	84	*D. sargus*	⬆Pase, AA ▬LiA	(Guerreiro et al., 2015b)
XOS	0.5%	FWP	56	*A. ocellatus*	⬆WG, SGR ⬇FCR ▬IFH, IFW	(Hoseinifar et al., 2016a)
XOS	1 %	FWP	56	*A. ocellatus*	⬆WG, SGR ⬇FCR ▬ IFH, IFW	(Hoseinifar et al., 2016a)
XOS	2%	FWP	56	*A. ocellatus*	⬆WG, SGR ⬇FCR ▬ IFH, IFW	(Hoseinifar et al., 2016a)
XOS		FWP	45	*Carassius auratus gibelio*	⬆WG, SGR, Pase, AA, LiA	(Xu et al., 2009)
XOS	0.05 - 0.6 %	FWP	56	*C. idella*	⬆WG, SGR ▬FCR, HSI, VSI, CF, IFN, IFH, IFW, SM, TG, CHO.	(Zhang et al., 2020)
XOS	0.002-0.010%	FWP	60	*C. idella*	⬆WG, SGR, VSI,	(Sun et al., 2021)
XOS	0.05 %	FWP	56	*O. niloticus* × *O. aureus*	⬆WG, AA, Pase ⬇FCR	(Poolsawat et al., 2021)
XOS	0.1%	FWP	56	*O. niloticus* × *O. aureus*	⬆WG, AA, Pase, IFH ⬇FCR	(Poolsawat et al., 2021)

XOS	0.2%	FWP	56	*O. niloticus* × *O. aureus*	⬆WG, AA, Pase, IFH ⬇FCR	(Poolsawat et al., 2021)
XOS	0.4%	FWP	56	*O. niloticus* × *O. aureus*	⬆WG, AA, Pase, IFH, IFW ⬇FCR	(Poolsawat et al., 2021)
XOS	0.6%	FWP	70	*P. gigas* × *P. hypophthalmus*	⬆ Pase, AA, IFH, ▬WG, SGR, HSI, VSI, TVC, HF, LiA ⬇FCR	(Hahor et al., 2019)
XOS	0.5-3%	FWP	56	C. carpio	⬆WG, PER, SGR, FCR, HIS, VSI	(Abasubong et al., 2018a)
XOS	2.5 g/kg	FWP	56	*O. mykiss*	▬WG, FCR, LiA, BC, IFW ⬆AA, IFH, MT, CD ⬇SGR	(Wang et al., 2022)
XOS	5.0 g/kg	FWP	56	*O. mykiss*	⬆ WG, SGR, LiA, AA, IFH, MT, CD ▬BC, IFW ⬇FCR	(Wang et al., 2022)
XOS	7.5 g/kg	FWP	56	*O. mykiss*	⬆ WG, SGR, LiA, AA, IFH, MT, CD ▬BC, IFW⬇FCR	(Wang et al., 2022)
XOS	10 g/kg	FWP	56	*O. mykiss*	⬆ WG, SGR, LiA, AA, IFH, MT, CD ▬BC, IFW ⬇FCR	(Wang et al., 2022)
XOS.	5 / 10 g/kg	FWP	56	*S. hasta*	▬BC, WG, SGR, FCR, PER, HF, LiA, Pase, AA	(Morshedi et al., 2019)
XOS	1%	FWP	49	*D. labrax*	▬WG, FCR	(Guerreiro et al., 2015a)
XOS	0.5-3%	FWP		*C. carpio*	⬆ Pase, LiA, CK , BMI, CF (Only 2%) ⬇FCR	(Abasubong et al., 2022)
XOS	0.5-2%	FWP	84	*M. amblycephala*	▬WG, FCR, HSI, VSI, CF, PER ⬇FCR	(Chen et al., 2022)

XOS.	5 / 10 g/kg	FWP	56	S. hasta	⬆ AA, Pase ▬WG, FCR, SGR, BC, LiA, PER	(Morshedi et al., 2020)
XOS	0.1%	FWP	56	*Carassius carassius*	⬆ WG, SGR, F1, PER	(Liu et al., 2022)

Abbreviations: AXOS-arabinose-xylooligosaccharide, XOS-Xylooligosaccharide, FWP-feed without XOS supplement, WG-Weight gain, SGR-Specific growth rate, FCR-feed conversion efficiency, PER-Protein efficiency rate, GA- Glycolytic activity, Pase- Protease activity, AA- Amylase activity, LiA-Lipase activity, TG-triglyceride, CHO-cholesterol, IFN-intestinal fold number, IFH-intestinal fold height, IFW -intestinal fold width, SM -submucosa, MT- muscular thickness, CD- crypt depth, BC - Body composition (protein, lipid & dry matter), HF -Haematological factors (Blood cells, Haematocrit, Haemoglobin concentration), VSI -Viscerosomatic index, HSI-Hepatosomatic index, CK-creatine kinase, BMI-body mass index, CF-condition factor.

Key: ⬆-significantly increased by XOS compared to control, ⬇- significantly decreased by XOS compared to control, ▬ - no significant difference between XOS supplementation and the control

Effects of dietary XOSs on immune system parameters

The immune system in Teleost fish responds to infection via cellular and humoral responses and comprises an innate immune system and adaptive immune system (Carbone and Faggio, 2016). Non-specific immune mechanisms in fish include lysozymes, complement systems, respiratory activity, phagocytic activity, and cytokines (Hoseinifar et al., 2015; Carbone and Faggio, 2016; Nawaz et al., 2018). In fish, innate immunity is the primary defence against diseases caused by pathogens and toxins. Numerous studies have reported using prebiotics to improve the non-specific immune responses of fish. As shown in Table 3, application of XOSs can modulate innate and humoral immunity in fish, resulting in improved mucosal integrity, immunity, and disease resistance. Collectively, studies have reported that dietary XOSs can significantly increase phagocytic activity, plasma nitric oxide activity, respiratory activity, myeloperoxidase content, catalase activity, total superoxide dismutase activity, glutathione peroxidase activity, phenoloxidase activity, and malondialdehyde concentration (Table 3). However, the prebiotic effects of XOSs on some innate immunity parameters tended to be inconsistent, with some showing no significant differences compared with the control one (Table 3). For example, in most studies, XOSs significantly improved the innate immunity indicated by serum lysozyme activity (Table 3).

Total immunoglobulin levels, erythrocyte counts and leucocyte cell counts were among the immunological parameters found to be the most inconsistent between the studies (sometimes affected and sometimes not) (Table 3). In most studies, dietary XOSs significantly increased complement levels (Table 3). Fish showed varied immune response parameters among species, possibly because fish have different beneficial microbiota communities. Microbial modulation by prebiotics results in variations in communities that have distinct effects due to changes in host-microbe interactions, which are mostly mediated by PRRs and their related microbial PAMPs (Nayak, 2010; RingØ et al., 2010; Hoseinifar et al., 2015). These

host-microbe interactions impact the immunological and regulatory responses of the host at both a localised and systemic level (Merrifield and Rodiles, 2015; Rawling et al., 2021). Some innate immune system parameters did not change after the challenge testing. The time-dependent sampling of immunological and antioxidant parameters requires further investigation.

SCFAs have also been shown to modulate innate, humoral, and molecular immunity of fish. However, the mode of action of SCFAs during direct XOS fermentation by beneficial bacteria in the fish gut has not yet been fully elucidated. As described in a review by Hoseinifar et al. (2016b), researchers have attempted to use SCFAs as feed additives and explore their modes of action on innate immunity in fish. SCFAs used as feed additives increased phagocytosis index, lysozyme activity, and mucosal immunity-related genes (*IL1-β, TNF-α,* and *TGF-β*) in fish (Hoseinifar et al., 2016b). Moreover, it has been shown that SCFA-binding G protein-coupled receptors are mainly expressed in innate immune cells and have been suggested as a possible mechanism for the effects of SCFAs on mammalian immunity (Deng et al., 2021; Li et al., 2022).

There has been little research into the effects of dietary XOSs on fish intestinal immune gene expression. Wang et al. (2022) observed that dietary XOSs supplementation (0.75% and 1%) increased *IL-10* gene expression while decreasing *TNF-α* and *IL-6* gene expression in triploid *Oncorhynchus mykiss* intestinal tissue. According to Wang et al. (2022), *claudin-1* and *ZO-1* gene expression increased substantially when *O. mykiss* fish were fed 7.5-10.0 g/kg XOS. This suggests that dietary XOSs supplementation could enhance the expression of tight junction membrane protein genes, increasing the integrity and stability of the intestinal mucosal barrier. Wang et al. (2022) reported that dietary XOSs supplementation decreased the expression of proinflammatory genes, *TNF-α* and *IL-6* in *O. mykiss* intestines. Furthermore, Abasubong et al. (2022), observed that dietary XOSs increased the expression of antioxidant and immune genes (*IL-1β, IL-8, TNFs, caspase-3 and caspase-9*) involved in innate immune response in the intestines of *Cyprinus carpio* (Table 3), potentially improving pathogen resistance. Liu et al. (2022) observed that dietary XOSs increased the expression of *TGF-β* and *IL-10* genes while decreasing the expression of *TNF-α, HSP90, IL-1β, TLR4, MyD88* genes in *Carassius auratus* intestines. Taken together, these studies suggest that dietary XOSs have the potential to enhance and sustain gut mucosal integrity by upregulating tight junctions and modulate immune functions through modulation of cytokine gene expression.

Table 3. Effects of dietary XOS on antioxidant and immunity parameters and gene expression

Intervention, Dosage/Source	Dose	Control	Duration (Days)	Fish species	Outcome	References
XOS		FWP	49	*D. labrax*	⬆TSD, CA, GPA, GA ⬇LA	(Guerreiro et al., 2015b)
Corncob derived-XOS produced using	1%	FWP	84	*O. niloticus*	⬆LA, GPA, PA, CC, PHA, ▬RBA	(Van Doan et al., 2020)

crude xylanase from *Aspergillus niger*.			56		⬆LA, GPA, PA, CC, PHA, RBA, SLA	
			28		⬆LA, GPA, PA, CC, PHA, RBA ▬ SLA	
Corncob derived-XOS produced using crude xylanase from *A. niger*.	0.5%	FWP	56	*O. niloticus*	⬆LA, GPA, PA, CC, PHA, RBA, SLA	(Van Doan et al., 2018)
			28		⬆LA, GPA, PA, CC, PHA, RBA, SLA	
Corncob derived-XOS produced using crude xylanase from *A. niger*.	1%	FWP	56	*O. niloticus*	⬆LA, GPA, PA, CC, PHA, RBA, SLA	(Van Doan et al., 2018)
			28		⬆LA, GPA, PA, CC, PHA, RBA, SLA	
Corncob derived-XOS produced using crude xylanase from *A. niger*.	2%	FWP	56	*O. niloticus*	⬆LA, GPA, PA, CC, PHA, RBA, SLA	(Van Doan et al., 2018)
			28		⬆LA, GPA, PA, CC, PHA, SLA ▬RBA	
XOS	0.5%	FWP	56	*M. amblyce phala*	⬆LA, TSD, MC, CA, TI, CC	(Abasubong et al., 2018a; Abasubong et al., 2018b; Abasubong et al., 2019)
XOS	1.5%	FWP	56	*M. amblyce phala*	LA (at 45 h but ▬ at 96 h), TSD, MC, CA, TI, CC	(Abasubong et al., 2018a; Abasubong et al., 2018b; Abasubong et al., 2019)
XOS	2.3%	FWP	56	*M. amblyce phala*	⬆LA, TSD, MC, CA, GPA (45 H and decrease but no significant effect at 96 h), TI, CC	(Abasubong et al., 2018a; Abasubong et al., 2018b; Abasubong et al., 2019)
XOS	3%	FWP	56	*M. amblyce phala*	⬆LA, TSD, MC, CA, TI, CC	(Abasubong et al., 2018a; Abasubong et al., 2018b;

						Abasubong et al., 2019)
XOS	0.5%	FWP	56	*S. hasta*	▬LA, TI, CC,	(Morshedi et al., 2018)
XOS	1%	FWP	56	*S. hasta*	▬LA, TI, CC	(Morshedi et al., 2018)
Corncob derived-XOS produced using Endoxylanase (PoXyn2) *P. occitanis* expressed in *P. pastoris.*	0.5%	FWP	84	*D. labrax*	⬆ LA (both pre-and post-challenge), TI (both pre-and post-challenge)	(Abdelmalek et al., 2015)
			56	*D. labrax*	⬆LA (both pre-and post-challenge), TI (both pre-and post-challenge)	(Abdelmalek et al., 2015)
Corncob derived-XOS produced from Endoxylanase (PoXyn2) *P. occitanis* expressed in *P. pastoris.*	1%	FWP	84	*D. labrax*	⬆LA (both pre-and post-challenge), TI (both pre-and post-challenge)	(Abdelmalek et al., 2015)
			56	*D. labrax*	⬆LA (both pre-and post-challenge), TI (both pre-and post-challenge)	(Abdelmalek et al., 2015)
XOS		FWP	84	*D. sargus*	⬆LA, MC, NOA ▬TI	(Guerreiro et al., 2015b)
XOS	1%	FWP	30	*D. labrax*		(Guerreiro et al., 2018b)
XOS		FWP	165	*D. labrax*	⬆LA ▬ TI, CC	(Azeredo et al., 2017)
XOS	0.05 %- 0.6%.	FWP	56	*C. idella*	⬆LA ▬BC, TSD ⬇ALT, ALP, AST, MDA	(Zhang et al., 2020)
XOS	0.05 %	FWP	56	*O. niloticus* × *O. aureus*	⬆ACP, TSD	(Poolsawat et al., 2021)

XOS	0.1%	FWP	56	O. niloticus × O. aureus	⬆ALP, ACP, TSD	(Poolsawat et al., 2021)
XOS	0.2%	FWP	56	*O. niloticus* × *O. aureus*	⬆ALP, ACP, TSD, CA	(Poolsawat et al., 2021)
XOS	0.4%	FWP	56	*O. niloticus* × *O. aureus*	⬆ALP, LA, TSD, CA	(Poolsawat et al., 2021)
XOS	0.6%	FWP	70	*P. gigas* × *P. hypophthalmus*	⬆TPC, LA, TI	(Hahor et al., 2019)
XOS	0.002 % - 0.010 %	FWP	60	*C. idella*	⬆Acetate, Propionate, Butyrate,	(Sun et al., 2021)
XOS.	5 and 10 g/kg	FWP	56	*S. hasta*	▬ TI, HF ⬆LA. There was no change in terms of the expression of *IL-Iβ* gene compared to the control group.	(Morshedi et al., 2020)
XOS	2.5 g/kg	FWP	56	*O. mykiss*	There was no change in terms of gene expression of intestinal *TNF-α, IL-10, IL-6 Claudin-1* and *ZO-1* compared to the control group.	(Wang et al., 2022)
XOS	5 g/kg	FWP	56	*O. mykiss*	Increased the gene expression of *claudin-1* and *ZO-1*. There was no change in terms of the expression of intestinal *TNF-α, IL-10* and *IL-6* genes	(Wang et al., 2022)

					compared to the control group	
XOS	7.5 g/kg	FWP	56	*O. mykiss*	Increased the gene expression of *IL-10*, *claudin-1* and ZO-1 and decresed the expression of *TNF-α* and *IL-6* genes	(Wang et al., 2022)
XOS	10 g/kg	FWP	56	*O. mykiss*	Increased the gene expression of *IL-10*, claudin-1, ZO-1 anddecrease the gene expression of *TNF-α* and *IL-6* genes in the intestines.	(Wang et al., 2022)
XOS	1%	FWP	49	*D. labrax*	▬GPx, LPO, FCR ⬇SOD, CAT	(Guerreiro et al., 2015a)
XOS	0.5-3%	FWP		*C. carpio*	⬆SOD, CAT, GPx, TI ⬇AST, ATP, MDA.	(Abasubong et al., 2022)
					IL-1β, IL-8, TNFs, Caspase-3, caspase-9, SOD, GPX, Lysosome-C, Compliment3, and mucin 5b immune genes in the intestine were upregulated.	
XOS.	5%/10 %	FWP	56	*S. hasta*	▬ LA, TI	(Morshedi et al., 2019)
XOS	0.1%	FWP	56	*Carassius carassius*	⬆SOD, CAT, GPx, ALP, ACP, LA, CC, TI⬇MDA	(Liu et al., 2022)
					The expression levels of *TGF-β* and *IL-10* genes were increased whereas the expression levels of *TNF-α*, *HSP90*, *IL-1β*, *TLR4* and *MyD88* genes were decreased.	

AXOS-arabinose-xylooligosaccharides, XOS-Xylooligosaccharides, FWP-feed without XOS supplement, LA- Lysozyme activity, CC- Complement content, PA- Phenoloxidase activity, TI- Total immunoglobulin, TSD- Total superoxide dismutase, MC- Myeloperoxidase content, CA-Catalase activity, GPA- Glutathione peroxidase activity, NOA- Nitric oxide activity, PHA- Phagocytosis activity,

RBA- Respiratory burst activity, GA- Glycolytic activity, Pase- Protease activity, SLA-Skin mucus lysozyme activity, ALP-alkaline phosphatase, ACP-Acid phosphatase, AST-aspartate aminotransferase, ALT-alanine aminotransferase, TG-triglyceride, CHO-cholesterol, MDA-malondialdehyde, Interleukin (IL), Tumour necrosis factor α (TNFα), LPO-lipid peroxidation, HF- haematological factors
Key: ⬆-significantly increased by XOS compared to control, ⬇- significantly decreased by XOS compared to control, ▬ - no significant difference between XOS supplementation and the control

Effects on disease resistance

Stimulating innate immunity by XOSs is considered a primary defence mechanism against opportunistic pathogens in fish. When the correct dosage, structure, and source of XOSs and the best probiotic(s) are used in the synbiotic formulation for fish, better protection against pathogens can be effectively achieved. In three studies, the dietary XOS significantly increased disease resistance in Nile tilapia and European sea bass (Table 4). In studies on Nile tilapia, dietary XOS significantly increased disease resistance to *Streptococcus agalactiae*, with survival rates of 55% and 60% in both studies (Table 4). Nile tilapia fed on a 10 g XOS/kg diet showed statistically significant relative survival rates and resistance to *Str. agalactiae* compared with the 5 g XOS/kg diet, 20 g XOS/kg diet and control groups (Table 4). In European sea bass, disease resistance to *A. hydrophila* was significantly increased by dietary XOS where fish fed with 10 g XOS/kg feed showed the most significant disease resistance to *A. hydrophila* (Table 4). Guerreiro et al. (2018a) suggested that the mechanism of prebiotics in disease resistance includes strengthening mucosal immunity and stimulating gut beneficial bacteria to prevent colonization by pathogenic bacteria. Other prebiotics, such as MOS and GOS, bind directly to mannose binding receptors of colonizing pathogenic bacteria and prevent them from occupying the mucosal epithelium (Guerreiro et al., 2018a). Dietary XOSs may also enhance resistance to diseases by stimulating innate immunity; however, any potential direct effects remain undocumented.

Further studies on the effects of the XOSs structure, dosage, and disease resistance in all fish species are required to develop appropriate dietary and therapeutic regimens. Differences between studies are most likely related to variations in the XOSs structure, feeding ratios, fish species, age/size, feeding management and duration, pathogen dose and virulence, and challenging methods. The success or potential of XOSs (and synbiotic applications of it) to prevent disease in many fish studies may be greater than the results shown owing to the use of the intraperitoneal (IP) technique for disease challenge. The IP approach avoids microbiota competition and by-passes the mucosal barriers; microbial competition with pathogens and enhanced mucosal barrier defences are key mechanisms by which prebiotics exert beneficial impacts and therefore IP challenges do not demonstrate the effects of feed additives on disease resistance but rather demonstrate their effects on infected fish (Merrifield et al., 2010 a).

Table 4. Effects of dietary XOSs on disease resistance in fish

Intervention	Dose	Control	Duration (Days)	Fish species	Outcome	References

Corncob derived-XOS produced using crude xylanase from *A. niger*.	1%	FWP	84	*O. niloticus*	⬆DR (56.25% vs. 31.25% in control, against *Str. agalactiae*).	(Van Doan et al., 2020)
Corncob derived-XOS produced using crude xylanase from *A. niger*	0.5%	FWP	56	*O. niloticus*	⬆DR (34.78%, against *Str. agalactiae*)	(Van Doan et al., 2018)
Corncob derived-XOS produced using crude xylanase from *A. niger*	1%	FWP	56	*O. niloticus*	⬆DR (60.87% against *Str. agalactiae*)	(Van Doan et al., 2018)
Corncob derived-XOS produced using crude xylanase from *A. niger*.	2%	FWP	56	*O. niloticus*	⬆DR (30.13% against *Str. agalactiae*)	(Van Doan et al., 2018)
XOS	0.5%	FWP	56	*M. amblycephala*	⬆DR (against *A. hydrophila*).	(Abasubong et al., 2018a; Abasubong et al., 2018b; Abasubong et al., 2019)
XOS	1.5%	FWP	56	*M. amblycephala*	DR (against *A. hydrophila*)	(Abasubong et al., 2018a; Abasubong et al., 2018b; Abasubong et al., 2019)
XOS	2.3%	FWP	56	*M. amblycephala*	⬆ DR (against *A. hydrophila*).	(Abasubong et al., 2018a; Abasubong et al., 2018b; Abasubong et al., 2019)
XOS	3%	FWP	56	*M. amblycephala*	⬆DR (against *A. hydrophila*).	(Abasubong et al., 2018a; Abasubong

						et al., 2018b; Abasubong et al., 2019)
Corncob derived-XOS produced using Endoxylanase (PoXyn2) *P. occitanis* expressed in *P. pastoris*	0.5%	FWP	84	*D. labrax*	⬆DR (Over 60% at 8 and 12 weeks) against *A. hydrophila*	(Abdelmalek et al., 2015)
			56	*D. labrax*	⬆DR (Over 60% at 8 and 12 weeks) against *A. hydrophila.*	(Abdelmalek et al., 2015)
Corncob derived-XOS produced using Endoxylanase (PoXyn2) *P. occitanis* expressed in *P. pastoris.*	1%	FWP	84	*D. labrax*	⬆DR (Over 60% at 8 and 12 weeks) against *A. hydrophila.*	(Abdelmalek et al., 2015)
			56	*D. labrax*	⬆DR (Over 60% at 8 and 12 weeks) against *A. hydrophila.*	(Abdelmalek et al., 2015)
XOS		FWP	84	*D. sargus*	▬BA	(Guerreiro et al., 2015b)
XOS		FWP	165	*D. labrax*	▬BA	(Azeredo et al., 2017)
XOS	0.05 - 0.6%.	FWP	56 days	*C. idella*	⬆DR against *A. hydrophila.*	(Zhang et al., 2020)
XOS	0.05 - 0.4%	FWP	56 days	*O. niloticus* × *O. aureus*	⬆DR against *A. hydrophila*.	(Poolsawat et al., 2021)
XOS	0.1%	FWP	56 days	tilapia O. niloticus × O. aureus	⬆DR against *A. hydrophila*.	(Poolsawat et al., 2021)
XOS	0.2%	FWP	56 days	*O. niloticus* × *O. aureus*	⬆DR (Against *A. hydrophila*)	(Poolsawat et al., 2021)

XOS	0.4%	FWP	56 days	*O. niloticus* × *O. aureus*	⬆DR gainst *A. hydrophila*.	(Poolsawat et al., 2021)
XOS.	5 and 10 g/kg	FWP	56	*S. hasta*	▬BA	(Morshedi et al., 2019)
XOS	0.1%	FWP	56	*C. carasius*	⬆DR (Against *A. hydrophila*)	(Liu et al., 2022)

XOS-Xylooligosaccharides, FWP-feed without XOS supplement, DR- Disease resistance, BA- serum bactericidal activity.

Key: ⬆-significantly increased by XOS compared to control, ⬇- significantly decreased by XOS compared to control, ▬ - no significant difference between XOS supplementation and the control

The dosage of XOS and feeding duration for better fish performance

As shown in Table 2, feeding fish on 1% XOSs often had a statistically significant positive prebiotic effects on the fish performance. The impacts of dietary XOSs dosing need to be continuously evaluated to determine the XOS dosage that will provide the most important benefits to each fish species while causing no toxicity. Inadequate dosing may explain some reported adverse effects of prebiotics on fish growth, immunity, and gut health. Therefore, XOSs dosing studies are critical for determining the best feeding guidelines for each fish species, age group and rearing system. The XOSs dosages and prebiotic effects reported to date have differed among the fish species, as shown in Tables 1-4. Several studies on Nile tilapia have shown that 1% of XOSs was the best dosage tested, followed by 0.5% and 2% dosages (Van Doan et al., 2018; Van Doan et al., 2020). Low dosages (0.1-0.7%) of XOSs were also tested in Nile tilapia, and XOSs was found to have health benefits at these low dosages (Tables 2-4). Finally, the optimal XOSs dose may vary depending on the XOSs structure and fish species, and there is a need to determine the correct XOSs structure and dosage for a specific fish species. Table 1 shows the prebiotic effects of XOSs on fish immunity, growth efficiency, and gut microbiota. Different fish species were fed different types and doses of XOSs for different durations. The minimum number of days the fish were fed was 28, and the highest was 165 days. Compared with the control, dietary XOSs supplementation resulted in a significant increase in some fish growth parameters.

XOS has been demonstrated to be nontoxic to fish based on results on liver function, renal function, and intestinal integrity. In all of the fish investigated, no toxicological results were identified and no negative impacts on feed consumption, feed conversion efficiency, haematology, clinical biochemistry, or organ weights have been identified. As a result, it is concluded that the high dosage level, at which the fish consumed approximately 3% XOS, exhibited no harm.

Synbiotic applications of XOS

Synbiotics, a combination of prebiotics and probiotics, can benefit the host by increasing survival and selectively promoting the development and activation of one or more health-promoting microbes, thereby improving host well-being (Gibson et al., 2010; Safari et al., 2017). Synbiotics can alter the host's intestinal microenvironment and modify or regulate the selective growth of beneficial microbes in adequate quantities, resulting in improved fish health. Few studies have been conducted on the effects of XOSs in synbiotic applications (i.e., probiotics + XOSs in combination as dietary supplements) in fish. However, Geraylou et al. (2013a) investigated applications of *L. lactis* spp. *Lactis* ST G45, *Lactococcus lactis* spp. *Lactis* ST G81, *Bacillus circulans* ST M53 and AXOS, either applied alone, or as a synbiotic application in a juvenile Siberian sturgeon (*A. baerii*) study. The authors reported a variety of interesting synbiotic effects where the combined use of the probiotic and prebiotic outperformed the individual prebiotic and probiotic application. For example, the only dietary treatment able to significantly improve growth performance parameters was the synbiotic application of *L. lactis* spp. *lactis* ST G45+AXOS. Statistical analyses of 16S rRNA libraries also revealed a synergistic effect of probiotic + AXOS for the relative abundances of number of OTUs. In addition, Van Doan et al. (Van Doan et al., 2020) reported that a synbiotic preparation of XOS derived from corn cob and *L. plantarum* CR1T5 improved growth performance, immunity, and disease resistance of Nile tilapia (*O. niloticus*) fingerlings against *Str. agalactiae*. Liu et al. (Liu et al., 2022) reported that dietary synbiotics of *B. subtilis* and XOS considerably increase crucian carp growth performance, antioxidant capacity, immunity, and resistance to *A. hydrophila*, and the combined effect was superior to the individual probiotic and prebiotic applications. However, the synbiotic effects of XOSs have not been fully explored. Therefore, XOS synbiotics must be evaluated to determine whether they are additive/complementary (their effects are similar to the sum of their independent effects) or synergistic (their effects are significantly greater than their individual beneficial effects). One method to increase the number of beneficial bacteria in fish intestine is to introduce strains in synbiotics rather than simply adding XOSs or probiotics. These synbiotics should ideally be XOSs-probiotic mixtures, with the probiotic preferentially digesting XOS. If synbiotics are designed such that dietary XOSs specifically supports the selective propagation of the probiotic, the probiotic has a better chance of colonising the intestinal mucosa of fish and competitively excluding pathogens already present in, or entering, the intestine.

In vitro and *in vivo* experiments have shown that probiotics and XOSs have a structure-function relationship that affects the fermentation rate and the number of metabolites that benefit the host (Aachary and Prapulla, 2011; Hoseinifar et al., 2017). Therefore, determining the structure of XOSs (short and long XOS chains, branched or unbranched) that have an optimal prebiotic effect on a particular fish species is critical. Furthermore, given the differential fermentability of XOSs, it is vital to understand the *in vitro* fermentation of XOSs by candidate probiotics. This should be a starting point for future *in vivo* research on the positive modulation of gut microbiota.

Conclusions and perspectives

Dietary XOSs have been documented to provide beneficial effects to some fish species, however, contradictory effects are present in the literature. The positive effects observed could

be attributed to increased digestive potential, improved intestinal barrier integrity, modulation of intestinal cytokine gene expression, enhanced innate immunity, improved disease resistance, and improved intestinal microbiota homeostasis. Conflicting results in some fish species could be due to the differences in fish species, the properties of XOSs, and the autochthonous host microbiota. Given the heterogeneity of the gut microbiome in different fish species, more comprehensive evidence using modern methods in a single fish species is needed to demonstrate the prebiotic effects of XOSs on the immune system, gut microbiota, and growth performance. In addition, a full systematic investigation of the *in vitro* fermentability of XOSs by gut-resident host bacteria or probiotics should be conducted before XOSs selection and administration, considering the composition, source, and production methods. Synbiotics applications including XOS/AXOS appear to be promising; however, many knowledge gaps exist and further studies are required.

Declaration of interests

The authors declare that they do not know about competing for financial interests or personal relationships that may affect the work reported in this paper.

Data Availability Statement

Data sharing does not apply to this article as no datasets were generated or analyzed during the current study.

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