



# Xylo-Oligosaccharides, Preparation and Application to Human and Animal Health: A Review

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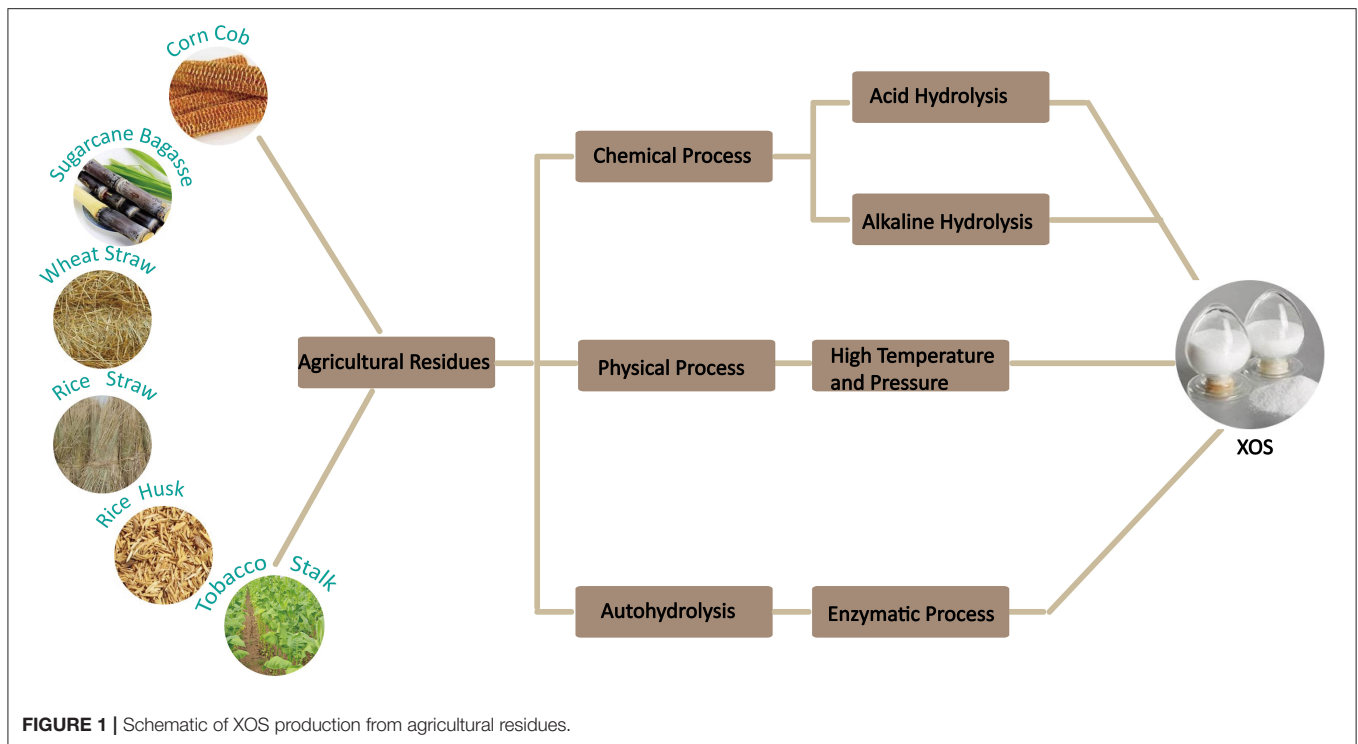
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Xylo-oligosaccharides (XOS) are considered as functional oligosaccharides and have great prebiotic potential. XOS are the degraded products of xylan prepared via chemical, physical or enzymatic degradation. They are mainly composed of xylose units linked by  $\beta$ -1, 4 bonds. XOS not only exhibit some specific physicochemical properties such as excellent water solubility and high temperature resistance, but also have a variety of functional biological activities including anti-inflammation, antioxidative, antitumor, antimicrobial properties and so on. Numerous studies have revealed in the recent decades that XOS can be applied to many food and feed products and exert their nutritional benefits. XOS have also been demonstrated to reduce the occurrence of human health-related diseases, improve the growth and resistance to diseases of animals. These effects open a new perspective on XOS potential applications for human consumption and animal production. Herein, this review aims to provide a general overview of preparation methods for XOS, and will also discuss the current application of XOS to human and animal health field.

**Keywords:** xylo-oligosaccharides, preparation, application, human health, animal health

## INTRODUCTION

During the few last decades, there is increasing interest in the use of nutraceuticals or functional food additives for improving human health which has led to development of new food and feed products during the last few decades (1). Many functional products, having prebiotic characteristics, such as xylo-oligosaccharides (XOS), fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), chito-oligosaccharides (COS), alginate-oligosaccharides (AOS) have been extensively used as food and feed additives (2–6). Among these prebiotics, XOS are considered to be very promising. XOS are the degraded products prepared by chemical, physical or enzymatic degradation of xylan derived from biomass materials such as sugarcane residues, corn cobs, rice straw, etc (7) (**Figure 1**). They are composed of xylose units linked by  $\beta$ -1, 4-xylosidic bonds, which have a branched structure by the addition of different side groups (Moreira et al.). The degrees of polymerization of XOS are usually 2–7 (**Figure 2**) and they are known as xylobiose, xylotriose, and so on (8).



XOS have a high potential to be applied for human nutrition due to its physicochemical properties such as low viscosity, high water solubility, tolerance to high temperature and acidic pH (9). Studies shown that XOS display a variety of pharmacological activities, including anti-inflammation, antioxidative, antitumor, antimicrobial properties. In addition, XOS have a potential application in the animal husbandry (10, 11). This review aims to summarize the methods of preparation of XOS and discuss the application of XOS to human and animal health.

## PREPARATION AND CHARACTERIZATION OF XOS

The most widely used preparation methods of XOS are: (1) chemical degradation methods (2) physical degradation methods and (3) enzymatic degradation methods (Figure 3).

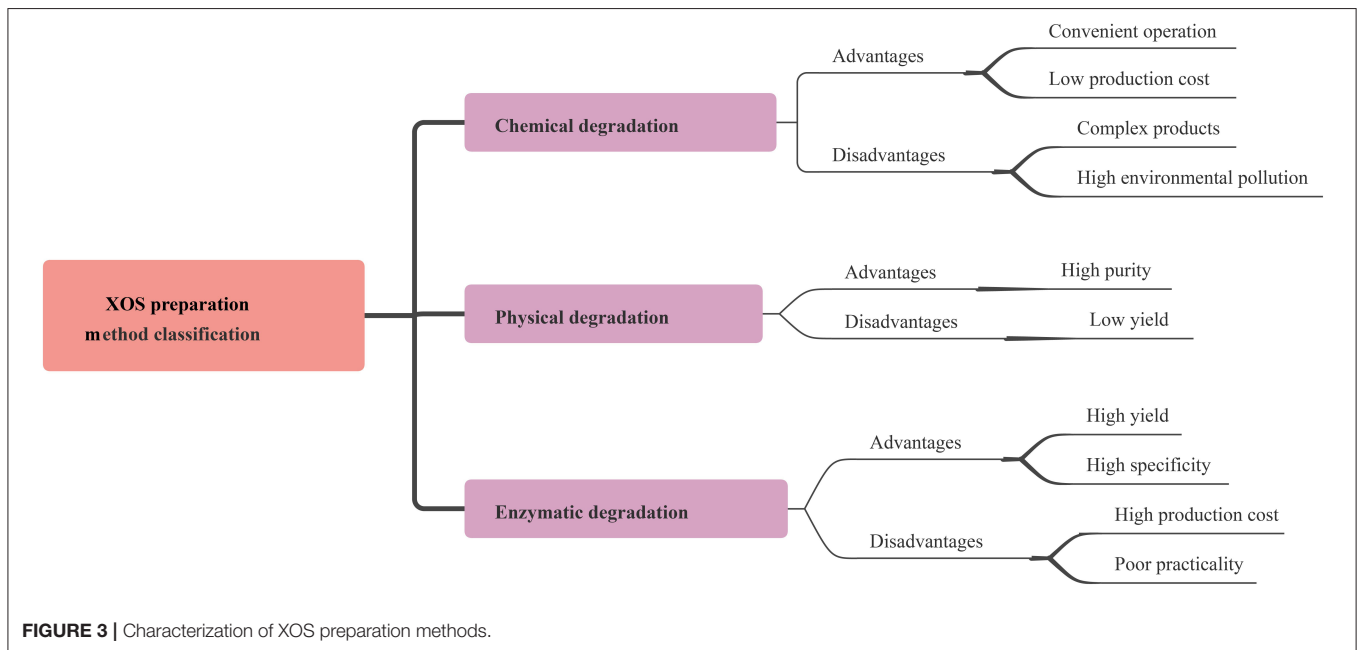
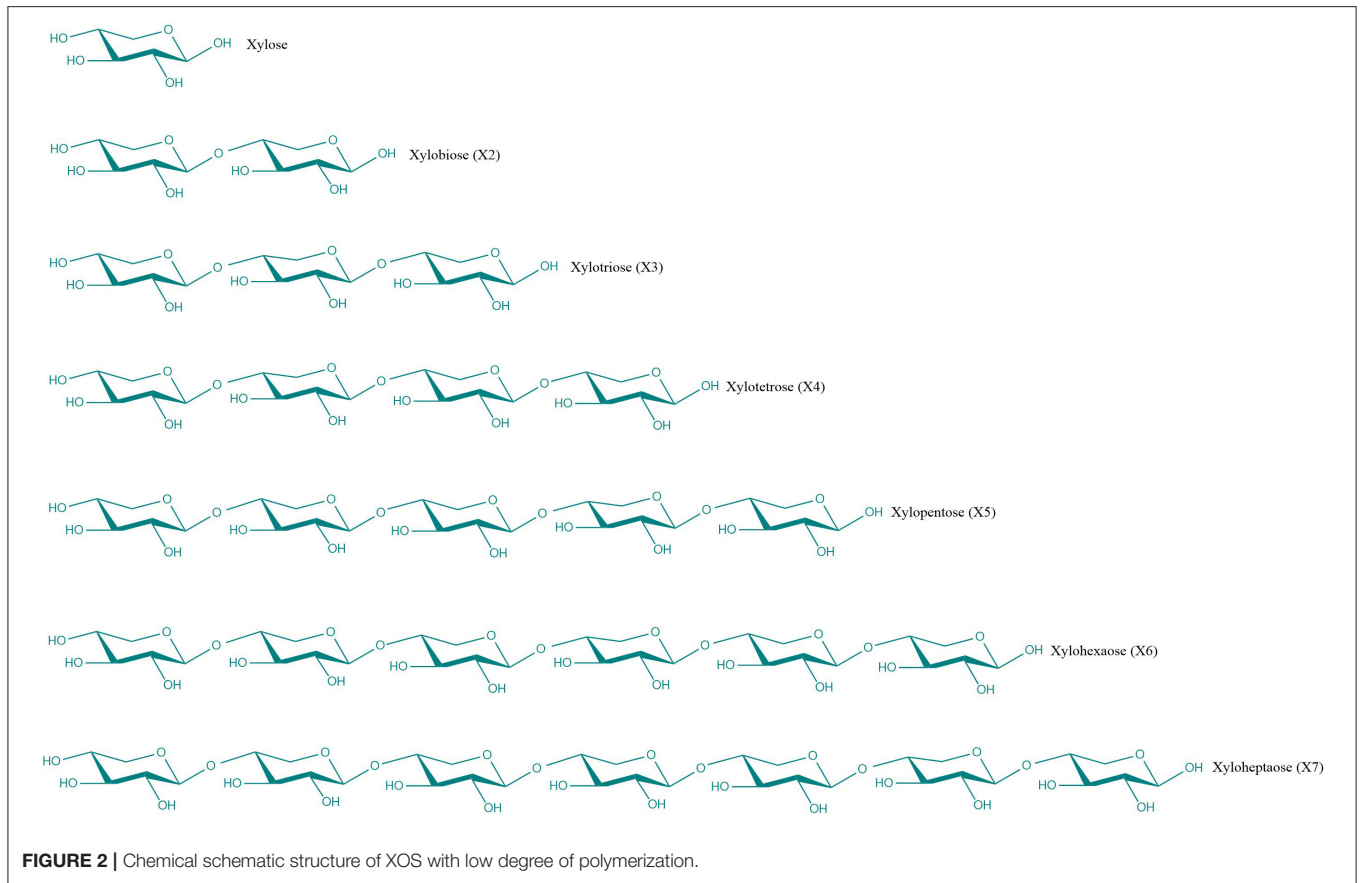
### Chemical Process for the Production of XOS

The chemical degradation process, especially the acid and the alkaline hydrolysis methods, has been widely used for the mass production of XOS in industry due to its advantages such as simple operation and low production cost. Several studies have been conducted on producing XOS with various inorganic acids (12–16). Samanta et al. reported that the xylan from tobacco stalks was hydrolysed by tartaric acid into XOS, mainly including xylobiose and xylotriose, in addition to monomeric xylose (16). XOS production can also be obtained from corn cob xylan using weak sulphuric acid at 90°C during 30 min (12). The production of XOS depends on both acid concentration and

hydrolysis time. A previous study showed that optimization of XOS production from waste xylan optimized by an orthogonal design of experiments, concluding a good extraction procedure of 20 min with 20% acetic acid at 140°C. A maximum XOS yield of more than 45.86% was obtained (14). Ying et al. studied that the increment of sulfuric acid concentration promoted the yield of xylooligosaccharides from hydrogen peroxide-acetic acid-pretreated poplar from 0.69 to 20.45% (17). In addition, Zhang et al. reported that acetic acid hydrolysis provided the highest XOS yield, up to 45.91% compared to hydrochloric acid and sulfuric acid pretreatment (15). It is widely known that the alkali solution could degrade hemicelluloses. This destruction is caused by the disruption of the hydrogen bonds with the alkaline reagent (18). In order to enhance the xylan content recovery from hemicellulose, use of appropriate alkaline concentration and pretreatment parameters are the primary conditions (19). For example, the use of higher concentration of alkali solution (15%) for extracting pineapple peels led to maximum recovery of hemicellulose. In the case of corn cobs, Samanta et al. also documented that higher concentration of alkali produced greater dissolution of hemicelluloses (12). However, these methods caused corrosion of the equipment, thus limiting their use.

### Physical Process for the Production of XOS

Production XOS products by physical degradation is relatively simple and environmentally friendly compared to chemical degradation. For example, XOS can be obtained from milled aspen wood using a microwave oven, processing at 180°C for 10 min were and nextly subjected to fractionation to oligo- and polysaccharides by size-exclusion chromatography. The



dispersion degree was smaller while the degradation effect was better (20). The hydrothermal reactor can also be used to degrade the xylan. Its fragments released from corn cob hemicellulose

are partially acetylated, which improves solubility of long xylo-oligosaccharides by preventing molecular interactions between the xylan and the main chains of the xylo-oligosaccharide

**TABLE 1** | Summary of XOS preparation and yields in the most recent studies.

Substrate	Pretreatment	Biocatalyst	Yxylan/ biomass (%)	Yxos/ biomass (%)	Yxos/ xylan (%)	DP	References
Corn cobs	acetic acid pH 2.7, 150°C, 30 min		30.4%	13.97%	45.9%	X2-X6	(14)
	Dilute acid followed by 135°C for 30 min	Xylanase from <i>Penicillium corylophilum</i> P-3-31	34.8%	23.6%	67.7%	X2-X4	(26)
	pH 6.5 and 60°C	Xylanase (PbXyn10A)	31.2%	23.4%	75%	X2-X4	(27)
	ultra-high pressure pretreatment	<i>Streptomyces thermovulgaris</i> TISTR1948 endoxylanase	33.4%	3.6%	10.7%	X2-X4	(28)
	190°C, 13 min	GH10 xylanase	29.9%	14.8%	49.4%		(21)
	5% (w/v) KOH, 90°C for 1 h		38.8%	11.5%	29.6%	X2-X5	(29)
Sugarcane Bagasse	Alkaline 10% (w/v) at room temperature overnight	Endo- $\beta$ -1,4-xylanase rHlxyn11A	10.5%	6.0%	57.4%	X2-X3	(30)
	15% (w/v) aqueous ammonia	$\beta$ -xylosidase	28.40%	19.3%	68.0%	X2-X4	(31)
	0.24M dilute H <sub>2</sub> SO <sub>4</sub> 90°C 31 min		33.5%	9.7%	29%	X2-X6	(13)
	5% gluconic acid hydrolysis (w/v) 60 min at 150°C	Cellulase	26.5%	14.1%	53.2%	X2-X6	(32)
	10% acetic acid at 150°C for 45 min	<i>G. oxydans</i> ATCC 621H	27.9%	10.9%	39.1%	X2-X6	(33)
Wheat straw	2% NaOH at 80°C for 90 min	The endoxylanase-variant K80R	8.4%	3.3%	39.8%	X2-X3	(34)
	Hydrolysis at 50°C and pH 5 for 5 h	$\beta$ -1,4-endoxylanase			44%	X2-X3	(35)
	180°C 40 min	Endo- $\beta$ -1-4-xylanase	73%	23%	31.5%	X2-X3	(36)
Rice straw	2% w/w sulfuric acid, 100°C, 0.5 h		65.3%	18.2%	27.8%		(37)
Rice husk	12% w/v NaOH, 110–120°C for 30 min	$\beta$ -1,4-xylanase	54.5%	9.5%	17.4%	X2-X5	(38)
Pineapple peel	15% (w/v) alkali solution for 16 h at 45°C, 50°C, pH 5.0 and 15 U enzyme dose	Endo- 1, 4-Xylanase M1		23.5%	25.7%	X2-X3	(39)
Finger millet seed coat	Sodium acetate	Xylanase of <i>Thermomyces lanuginosus</i>	4.8%	3.4%	71.8%	X2-X3	(40)
Tobacco stalk	8% KOH or NaOH 90°C, 1M tartaric acid		17.0%	6.1%	35.7%	X1-X3	(16)

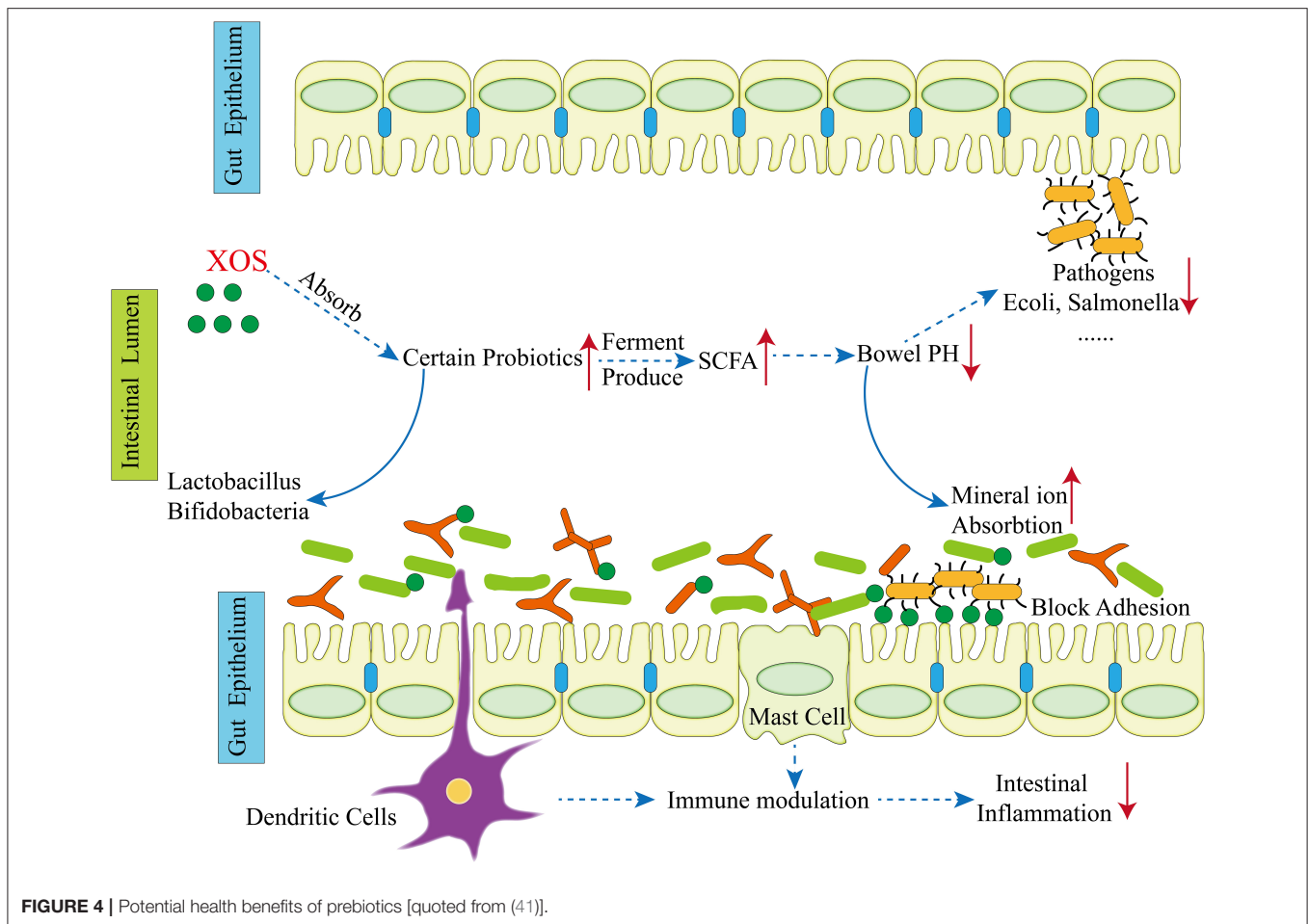
and also by preventing the binding of xylan to cellulose (21). The purity of XOS products is relatively high from physical degradation. However, there is limitation on the use of this method for large-scale production of XOS due to low yield.

## Enzymatic Process for the Production of XOS

The industrial process of XOS production from natural xylan-rich agricultural residues involve enzymatic hydrolysis. As compared to the acid and alkaline hydrolysis method, production by the enzymatic degradation is relatively more economical, quick, and eco-friendly. Furthermore, enzymatic hydrolysis neither requires any special equipment nor produces undesirable byproducts. Thus, the production of XOS by enzymatic means was done from plant sources rich in xylan including corn cobs, sugarcane bagasse, wheat bran, birch wood, oat spelt, beech wood, natural grass, oil palm frond etc. These major enzymes used include  $\beta$ -xylosidase, glycosynthases and endoxylanases, the latter being the key enzyme to produce XOS from xylan. They are able to reduce monomeric xylose release

from the non-reducing ends of xylooligomers and xylobiose. The endo-xylanases from families GH10, GH11, and GH30 act specifically on the substituted and unsubstituted regions of xylan chain (22). Other studies focused on the use of  $\beta$ -xylosidases and glycosynthases for XOS production.  $\beta$ -xylosidases catalyze substrate hydrolysis by inversion or retaining mechanism and are classified into six GH families: GH3, 30, 39, 43, 52, and 54. The  $\beta$ -xylosidases have been reported to produce longer  $\beta$ -XOS from  $\beta$ -1, 4 linkages or synthesize novel XOS (23, 24). Kim et al. documented that a glycosynthase derived from a retaining xylanase could synthesize a great variety of XOS (25). Many factors affect the yield of XOS from xylan such as the enzyme activity, the raw material, and incubation conditions including incubation pH, reaction time and temperature (19).

**Table 1** summarizes the preparation process and the yields of XOS produced from xylan and xylan biomass by different approaches, often leading to high yields for several sources of substrates. Importantly, the prebiotic action of XOS requires a low degree of polymerization (DP) (9, 18). Hence, there are still some parameters in the preparation process of XOS that need to be optimized, including the production of a low DP (DP of 2–7) and the achievement of a high purity. Therefore, research



focuses on the combination and integration of the processes, testing different raw materials, extraction methods and enzymes to achieve an economically viable and health promoting product with an optimal production efficiency.

## XOS APPLICATION TO HUMAN HEALTH

XOS were demonstrated to have various activities in human health such as inducing immune modulation, anti-tumor, antioxidant and anti-microbial effects (**Figure 4**).

### Immune Modulation Effects of XOS

It is essential for protecting the host from diseases or repairing tissue injury to release inflammatory mediators (42, 43), and XOS is thus suggested to be an immunomodulator to prevent adverse immune-related conditions. Indeed, XOS was shown to have immunomodulatory effects by regulating expression of several proinflammatory mediators *in vitro*. XOS not only suppressed TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and NO expression, but also triggered IL-10 production in lipopolysaccharide (LPS)-stimulated RAW264.7 cells (44). XOS feeding significantly decreased expression of IL-1 $\beta$  and IFN- $\gamma$  and attenuated systemic inflammation (45).

Moreover, the O-acetylated XOS derived from almond shells and their deacetylated derivatives exhibited immunomodulatory potential, based on a mitogenic rat thymocyte test (46). Finally, XOS combined with inulin attenuated the expression of IL-1 $\beta$  in the blood of healthy subjects fed a high-fat diet (47). Schematic presentation of XOS health benefits and their role in immune modulation are depicted in **Figure 4**.

### Anti-tumor Effects of XOS

The main causes of cancer are the uncontrolled proliferation of abnormal cells which may stay at the point of mutation or metastasize into other locations. It has been shown that XOS exposure showed effect in preventing cancer (48–50). Indeed,  $\beta$ -1,3-Xylooligosaccharides with an average DP of 5 extracted from green alga *Caulerpa lentillifera* inhibited the number of viable human breast cancer MCF-7 cells in a dose-dependent manner, and induced apoptosis (50). Thus, this XOS could be a promising agent for prevention of breast cancer. Moreover, XOS supplementation reduced the level of lipid peroxidation and increased the activities of glutathione-S-transferase and catalase in colonic mucosa and liver, which may have contributed to the inhibition of colon carcinogenesis (51). *In vitro* approaches will

be useful for future mechanistic characterization of the antitumor properties of XOS. However, no systematic attempts have been carried out to study the upstream signals of caspase activation and the specific effects *in vivo*. Further research is necessary to investigate the overall anti-tumor effect of XOS.

### Antioxidant Effects of XOS

During both acute and chronic diseases in humans, the abundance of free radicals usually increases. Several notable studies demonstrated that XOS had exhibited strong antioxidant and free radical scavenging activity, thus suggesting a potential use in biomedical applications (52, 53). The scavenging ability of XOS was shown to be dose-dependent (54), and this potential is likely attributable to efficient release of phenolic compounds and transfer of hydrogen atoms from the phenolic compounds to free radicals (55). Jagtap et al. revealed that the percent of antioxidant activity gradually increased reaching the maximum, 74% at a concentration of 6 mg/ml XOS using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay, after which it did not show any further increase (56). Bouiche et al. studied that the antioxidant activity of glucuronoxyloligosaccharides (UXOS) and arabinoxyloligosaccharides (AXOS) was tested with the 2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method (57). The results showed that the antioxidant activity of UXOS was significantly higher than the antioxidant activity of AXOS. Although both have neutral molecules, UXOS also has methylglucuronic acid (MeGlcA) decorations that confer a negative charge to the XOS. It was assumed that the MeGlcA decorations of the XOS were key elements influencing their antioxidant and radical scavenging activity of XOS (58).

### Anti-microbial Effects of XOS

It has been reported that XOS have significant antimicrobial effects against several pathogenic bacterial. A host of clinically important both Gram-negative and Gram-positive bacteria have been documented to be sensitive to XOS exposure. Indeed, XOS and FOS supplementations markedly reduced the cecal pH level and increased the population of bifidobacterial compared with the control and DMH (1,2-dimethylhydrazine) treatments and the XOS treatment group had a lower abundance of *E. coli* than the DMH group. These results indicated that XOS and FOS non-digestible carbohydrates may promote the health of intestinal tract (59). In addition, some *in vitro* studies have documented that XOS supplementation produced lactic acid and acetic acid, which contributed to growth of *bifidobacteria* and *lactobacilli* strains and inhibited the growth of pathogenic strains (60–63).

## XOS APPLICATION TO ANIMAL HEALTH

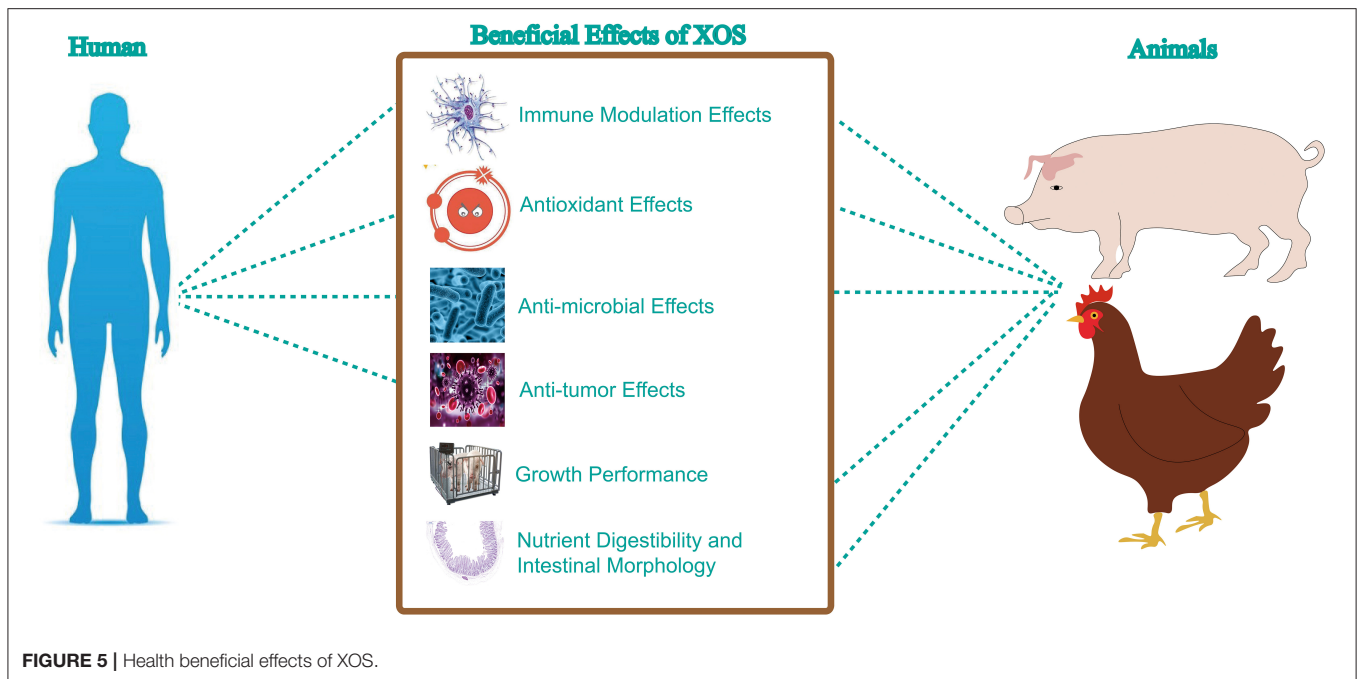
In this section, the recent studies on the application of XOS in animal husbandry health are provided. We have noted that most of the studies were focusing on XOS modulation of growth performance, nutrient digestibility and intestinal morphology, immune and anti-oxidant activity and gut microbiome (Figure 5).

### Effects of XOS on Growth Performance of Animals

XOS have been used for animal nutrition and health improvement due to their potential biological functions, such as antioxidant, anti-inflammatory and antimicrobial effects. Previous studies have demonstrated the benefits of XOS on the growth performance of animals. Liu et al. reported that XOS treatment at a dose of 200 mg/kg increased average daily gain (ADG) by 17% and gain to feed (G/F) by 14% in the whole experiment, improved the apparent total tract digestibility (ATTD) of dry matter (DM), N and gross energy (GE) during 0 to 14 d in the piglets (27). Our study found that the effects of 500 mg/kg XOS (XOS500) on the growth performance during 1–28 days were very similar with that of the antibiotic chlortetracycline in the piglets. The results showed that XOS500 (500 mg/kg XOS) supplementation could significantly increase body weight (BW), ADG, average daily feed intake (ADFI) and feed to gain (F: G) of piglets (64). However, another study failed to notice significant improvement on growth performance after 0.01% XOS treatment in pigs (65). The discrepancy might be caused by the different levels of XOS used in these studies. Thus, further studies are needed to confirm the optimal dose of XOS in pigs. In addition, Yuan et al. evaluated the effects of XOS on growth performance and immune function of broiler chickens. They reported that XOS supplementation in the diet of broiler chickens significantly improved ADFI and ADG at 1–42 days when compared to the control group (66). The results of a study by Pourabedin et al. demonstrated that the feed conversion ratio (FCR) in broilers fed 2 g XOS/kg diet was lower than those fed 1 g XOS/kg diet between days 7 and 21, which is in line with other studies (67, 68). Some other researchers found that the FCR in the control group was also significantly lower for the group receiving the XOS-supplemented diet in broiler chickens for the whole trial period (67, 68). These results showed that XOS may dose-dependently improve the growth performance of animals and have potential as novel alternatives to antibiotics as growth promoters.

### Effects of XOS on Nutrient Digestibility and Intestinal Morphology of Animals

The growth promoting effect of XOS has been shown to be related to improvement in nutrient digestibility. The addition of 200 mg/kg XOS with a purity of 50% supplementation has been demonstrated to improve the apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N), and gross energy (GE) in weaning pigs on d14 (11). Similarly, the XOS supplementation significantly increased the apparent digestibility of the calcium with the increasing concentration of dietary XOS (0.1, 0.2, 0.3, 0.4 or 0.5 g/kg) in laying hens (69). The improvement of nutrient digestibility may be the result from XOS supporting normal intestinal morphology. Intestine morphology indices are often as a useful criterion to estimate the nutrient digestion and absorption capacity of the intestine. It is generally believed that the jejunum is the main segment involved in absorption of nutrients and minerals (70). Our study indicated that the XOS500 supplementation increased the villus height and villus height



to crypt depth ratio in the jejunum and ileum in comparison with the CON and XOS1000 group in the piglets, possibly improving nutrient absorption (71). Liu et al. confirmed that the XOS increased villus height to crypt depth ratio in jejunum, but did not influence villus height, crypt depth in the piglets (27). Similarly, Ding et al. reported that there was a linear improvement in villus height and villus height to crypt depth ratio of the jejunum as dietary XOS concentration increased in the laying hens (10). This is in agreement with the study of Maesschalck et al. showing that supplementation of 0.5% XOS with a purity of 35% to broiler chicken feed significantly increased the villus height in the ileum, suggesting an increase in gut health and improved nutrient absorption (68). However, 0.01% XOS with a purity of 40% in the diet of weaned piglets had little effects on the intestinal structure and villus surface area (65). In addition, the addition of 75 mg/kg XOS with a purity of 35% in the diet decreased the crypt depth of the duodenum (67). These results indicated that the use of an appropriate level of XOS may be important for increasing intestinal health and function.

### Effects of XOS on Immune Modulation and Anti-oxidant Activity of Animals

XOS have been reported to display significant anti-inflammatory and anti-oxidant activities in animals in previous studies. In pigs, Yin et al. reported that dietary XOS markedly reduced serum IFN- $\gamma$  concentration, indicating an anti-inflammatory effect of XOS (65), which is in line with a study in broilers showing a downregulation of the IFN- $\gamma$  gene mRNA expression of jejunal mucosa. In addition, an increase in plasma IgG concentration was observed in XOS-fed 21-day-old broilers (66). Furthermore, XOS increased plasma IgA, IL-2, and TNF- $\alpha$  concentration compared with the control diet, and

linearly improved the IgA and TNF- $\alpha$  concentration in plasma increasing the dietary XOS concentration in the laying hens (10). These results indicated that dietary XOS may improve cell-mediated immune response in early weaned piglets by regulating the production of cytokines and antibodies. In addition, antioxidant defense systems are regarded as important serum indices for assessing animal health. The changes in the antioxidant defense systems mainly including total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) may indicate oxidative stress (72). Several studies revealed that XOS had exhibited antioxidant and radical scavenging competency (73). However, the research of Guerreiro revealed that the XOS supplementation reduced antioxidant enzyme activities in European sea bass (74).

### Effects of XOS on the Modulation of Gut Microbiome of Animals

Our recent study showed that XOS500 supplementation could significantly increase the relative abundance of *Lactobacillus* genus and reduce the relative abundance of *Clostridium\_sensu\_stricto\_1*, *Escherichia-Shigella*, and *Terrisporobacter* genus in the ileum and cecum in piglets (64). Moreover, 200 mg/kg XOS administration decreased fecal *Escherichia coli* and increased *Lactobacilli* in piglets (11). However, dietary XOS reduced the relative abundance of the *Lactobacillus* and increased the relative abundances of *Streptococcus* and *Turicibacter* (65). Furthermore, XOS and GOS both markedly decreased the numbers of intestinal *Listeria monocytogenes* in ileal samples from guinea pigs, and selectively stimulated *bifidobacteria* and *lactobacilli*, which are believed to have inhibitory effects against pathogens (75).

Similar beneficial effects of XOS have been observed in broilers. Indeed, 2 g XOS/kg diet increased the relative abundance of the *Lactobacillus* genus in the cecal microbiota of broilers (76), that can adhere to the mucosa and epithelium, promoting colonization, immunomodulation and protecting the intestinal barrier against pathogens (77). Furthermore, by the production of lactate, the lower the intestinal pH, inhibiting the growth of acid-sensitive pathogenic bacteria (78). However, the specific effect mechanism of XOS on the gut microbiome remains unclear as several studies were only done (18–20) or by microbial culture methods (21) that fail to provide accurate taxonomic composition and community structure. Thus, extensive research will be required to determine effects of XOS on the microbiome in animals.

## CONCLUSION

In this review paper, we have summarized the preparation methods for XOS and its potential use as a functional food or feed additive for human and animal health. XOS seem to beneficially promoting intestinal health by selective stimulation of growth of *bifidobacteria* and *lactobacilli*. XOS also reduce the abundance of potentially pathogenic organisms. In addition, XOS exhibit a variety of biological activities including effects in suppressing inflammation, antioxidative, antitumor and antimicrobial properties. However, there are still several bottlenecks in the preparation and application of XOS. It is still difficult to obtain XOS products in large

scale with high purity, and lack of consistency in quality of different batches of XOS from different polymerization degrees due to a lack of standardized preparation methods. The XOS products in the market are mainly mixtures not monomers. Technologies should be developed for producing XOS monomers with high purity at low cost. In addition, new investigations are required to further elucidate the specific molecular mechanisms of XOS. Additional information is needed on the mode of absorption of XOS in the host after oral ingestion, and the identification of related receptors or responsible for the transportation of XOS into target cells. Progress in these areas may enhance the value of XOS for applications in the prevention and treatment of human diseases and animal production.

## AUTHOR CONTRIBUTIONS

YC, LC, and HZ wrote the first draft of the manuscript. NE, YB, and KA critically evaluated the manuscript. YX, RZ, and TL help check and revise the manuscript. All authors contributed to the article and approved the submitted version.

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**Conflict of Interest:** TL is employed by Hunan United Bio-technology Co.

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