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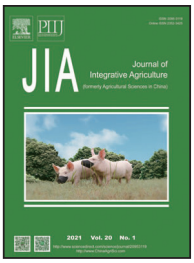

REVIEW

Alginate oligosaccharides preparation, biological activities and their application in livestock and poultry

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Abstract

Alginate oligosaccharides (AOS), belonging to the class of functional marine oligosaccharides, are low-molecular polymers linked by β-1,4-mannuronic acid (M) and α-1,4-guluronic acid (G), which could be classically obtained by enzymatic hydrolysis of alginate. With low viscosity and good water solubility, as well as anti-oxidant, immune regulation, anti-bacterial and anti-inflammatory activities, AOS have been widely used in medical science and functional food, green agriculture and other fields. As new bio-feed additives, AOS have broad potential applications in animal husbandry. In this review, the sources of alginate, chemical structure and preparation methods of AOS, and their biological activities and application in livestock and poultry are summarized. We expect this review could contribute to lay a foundation of application and further research for AOS in livestock and poultry.

Keywords: alginate oligosaccharides, preparation, anti-oxidant, immune, livestock and poultry

1. Introduction

Alginates, typically obtained from marine brown seaweeds, are linear and anionic polymers of α-L-guluronate (G) and their C5 epimer β-D-mannuronate (M), arranged as homopolymeric G blocks, M blocks, alternating GM or random heteropolymeric G/M stretches (Gacesa 2009; Lee and Mooney 2012; Kang *et al*. 2015). They have been extensively investigated and used in biomedical science and engineering due to their favorable properties of biocompatibility, low toxicity and relatively low cost (Wong *et al*. 2000; Wang *et al*. 2006). However, further application of AOS was restricted by their disadvantages, such asrelatively large molecular weight, high viscosity and poor water solubility. Alginate oligosaccharides (AOS), produced by depolymerizing alginates using different degradation methods including enzymatic degradation, oxidative degradation, and acid hydrolysis, are non-immunogenic, non-toxic, biodegradable polymer generally with low molecular weight within 1 000 Da (Wan *et al*. 1992; Guo *et al*. 2016). AOS as bioactive compounds resulting from alginates, have multiple biological activities such as anti-oxidant, anti-apoptotic, anti-inflammatory and anti-

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carcinogenic (Wang *et al*. 2007; Tusi *et al*. 2011; Zhou *et al*. 2015; Yang *et al*. 2017). However, further researches are needed on the use of AOS as a feed additive in livestock and poultry (Wan *et al*. 2017). In this paper, we mainly review the preparation process of AOS, and their biological activities and application in livestock and poultry.

2. Sources of alginate and chemical structure of AOS

Alginates are gelling polysaccharides and mainly present in the cell walls of marine brown algae (*Phaeophyceae*), such as *Macrocystis*, *Laminaria*, and *Ascophyllum* (Wong *et al*. 2000; Jacobsen *et al*. 2019). They are linear polysaccharides composed of β-D-(1,4)-mannuronic acid (M) and α-L-(1,4)-guluronic acid (G). The monomeric units of alginates, G and M, are C5 epimers of each other (Zhu and Yin 2015; Guo *et al*. 2016; Yang *et al*. 2017) (Fig. 1). The ratio of M to G in alginates varies according to algae, region, season and distribution in algae, which is roughly 2:1–1:2 (Johnson *et al*. 1997). The composition of alginates found in algae may differ by the environment and life cycle, and the older tissue is richer in G-blocks, and the frond tips in mannuronate.

The AOS rare linear oligomer linked with a 1,4-glycosidic bond with a degree of polymerization of 2–8 obtained by degrading alginates . These residues form homopolymeric segments in the molecule, such as polymannuronic acid (PM) formed by M through β-D-(1,4)-linkages and α-L-(1,4)-connected formation of polyguluronate (PG) by G, or three structural fragments of PMG segments alternately copolymerized with M and G (Wong *et al*. 2000; Matsushima and Minoshima 2005; Yamasaki *et al*. 2012).

3. AOS preparation

AOS are low molecular oligomers generally prepared from macromolecular alginates with methods of acid hydrolysis, oxidation and bio-enzymatic hydrolysis. The preparation methods are shown in Table 1, mainly including chemical, physical and/or enzymatic methods (Aida *et al*. 2010).

3.1. Chemical method

The chemical degradation methods include acid hydrolysis, alkaline hydrolysis and oxidative degradation, which are traditional ways for AOS production. The method of acid hydrolysis is the simplest and most effective at present (Yang *et al*. 2004; Lu *et al*. 2015). In an acidic environment, glycosidic bonds are easily cleaved under H^+ catalysis to form low molecular fragments of unequal degree of polymerization (Holtan *et al*. 2006). However, the acid degradation reaction is random and without specificity. According to the pKa difference between PM and PG (pKa of M is 3.38 and pKa of G is 3.65), the acid degradation method mainly produces a mixture of oligosaccharides with different acidic fragments. Different degrees of degradation of alginates can be made by controlling the reaction temperature and reaction time during the degradation. The drawbacks of the acid hydrolysis reaction are time-consuming (usually 4–12 h), and large amounts of salt will be produced from neutralizing the remaining acid with the alkali, which may interfere with subsequent AOS separation and analysis. Moreover, the resultant AOS product has a poor appearance in terms of color.

When alginic acid was treated with high concentrations of alkali, several products could be obtained, including glucuronide, anhydrous isogluconic acid and 2-deoxy-3-C-methyltetrahydro acid, whereas at low concentrations of alkali, 2,3-dideoxypentaric acid was the major product (Niemelä and Sjöström 1985). This suggested that alginic acid was directly degraded from the internal linkage when being treated with alkali.

The oxidative degradation method is to degrade polysaccharide by adding oxidizing agents to alginate solution. The commonly used oxidizing agents are H_2O_2, NaClO, $KMnO_4$ and $NaNO_2$. Yang *et al*. (2004) optimized their oxidative degradation conditions when using alginate-

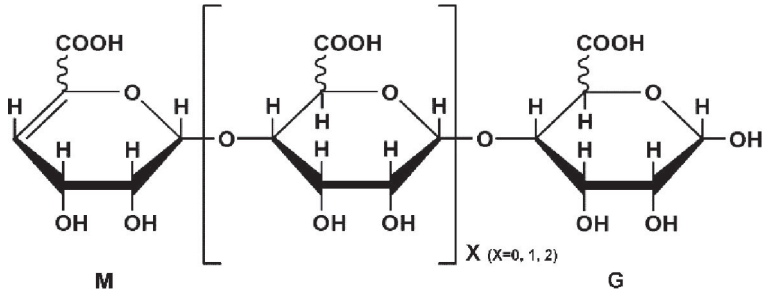

Fig. 1 Molecular structure of alginate oligosaccharides (AOS) (Guo *et al.* 2016). M, β-D-mannuronate; G, α-L-guluronate.

Table 1 A list of short protocols and preparation methods of alginate oligosaccharides (AOS)

Materials	Protocols	Methods	Ref.
Alginates	Alginates are hydrolyzed with 1 mol L^{-1} oxalic acid at 100°C, then hydrolyzed in 0.3 mol L^{-1} HCl solution for 2 h to form small molecular fragments of G, M and MG	Chemical-acid method	Haug *et al.* (1966)
Lessonia trabeculata	A total of 50 mg sodium alginates in 80% H_2SO_4, 20°C hydrolysis for 18 h, adding 1 mol L^{-1} H_2SO_4 at 100°C for 5 h for further hydrolysis, and adding $CaCO_3$ for neutralization, then using ion exchange chromatography to elute acetic acid 6 mg sodium alginates adding 5 mL 90% formic acid hydrolyzed at 100°C, adding 1.5 mol L^{-1} formic acid at 100°C for 2 h	Chemical-acid method	Nishide *et al.* (1984); Chandía *et al.* (2001)
Alginic acid	A total of 2 mol L^{-1} NaOH was added to 300 mg alginic acid, placed in the rotary cooker treating at 95°C and 135°C for 2.5 h, then added 0.1 mol L^{-1} $Ca(OH)_2$	Chemical-alkali method	Niemelä and Sjöström (1985)
Sodium alginates	A total of 6% H_2O_2 at 60°C for 8 h to yield AOS with a 60% productivity (1 000–8 000 Da)	Chemical-oxidative degradation	Li *et al.* (2015)
Sodium alginates	A total of 2.0 wt% sodium alginates solution was placed in the alloy tube, and the supercritical water was rapidly heated to 250–375°C (523–648 K) for hydrolysis for 17–88 ms	Physical-heating method	Matsushima and Minoshima (2005)
Sodium alginates (300–400 mPa s)	Sodium alginate solids (or 1.4% liquid) were irradiated with γ-ray of Co60 at 10^{-3} Pa at a dose rate of 10 kGy h^{-1}	Physical-radiation method	Aida *et al.* (2010)
Sodium alginates (less than 200-mesh power)	Culturing *A. macleodii* bacteria to prepare alginate lyase; 50 g sodium alginates are mixed with 133 mL alginate lyase and heated at 35°C, pH 7 for 20 h, and at 95°C for 15 min. The filtrate was concentrated to 70% to obtain the lysate that was separated by anion exchange column chromatography	Enzymatic method	Nagasawa *et al.* (2000)
Sodium alginates (average molecular weight 9 600±300; M/G=53/47)	Culturing *A. macleodii* bacteria to prepare alginate lyase; 50 g sodium alginates are mixed 133 mL alginate lyase and heated at 35°C, pH 7 for 20 h, and at 95°C for 15 min. The filtrate was concentrated to 70% to obtain the lysate, and the product was separated by anion exchange column chromatography	Enzymatic method	Natsume *et al.* (1994)
Sodium alginates	Culturing *Alteromonas* sp. 1786 to prepare the lyase; 0.5% sodium alginate and 0.05 mol L^{-1} sodium phosphate buffer added with lyase was incubated at pH 7.5, 40°C for 6 h, sodium ion and phosphate ion were removed by electrodialysis, and AOS are obtained by lyophilization	Enzymatic method	Kawada *et al.* (1997)
Sodium alginates (M/G=2.28)	Extracting lyase from *Vibro* sp. 510 fermentation broth; 5 g sodium alginates added with 50 mmol L^{-1} Tris-HCl buffer (pH 7.5, 50 U lyase) incubated at 28°C for 24 h, and at 100°C for 10 min, followed with ethanol solution to 50% (v/v). The supernatant was centrifuged and lyophilized to obtain AOS	Enzymatic method	Zhang *et al.* (2004)

derived PM as raw material to get the degradation product with 3–11 targeted polymerized oligosaccharide product and a selection criterion of 1 500 Da. When treated at 5% H_2O_2 and 90°C for 2 h, the oligosaccharides prepared by oxidative degradation still maintain their structural characteristics of PM as detected by circular dichroism and infrared spectroscopy. In another study, H_2O_2 was used to degrade alginate to prepare small molecular fucoidan of which the degradation rate of alginate was related to reaction time, temperature, solution pH and H_2O_2 concentrations (Li *et al*. 2010).

In summary, by controlling reaction conditions, chemical method can be used for degrading alginates, and thereby for producing high purity homogeneous oligosaccharides. Yang *et al*. (2004) used oxidative degradation method to prepare AOS with the proportion of 15.6, 26.7, 20.4, 14.8, 11.1, 8.4 and 3% for the 1, 2, 3, 4, 5, 6 and 7-mers, respectively, with a main population of 1–5-mers (88.6%). Although chemical methods have the advantages of low cost and mature technology system, still there are some problems including violent reaction, cumbersome operation steps, demanding corrosive-proof equipment and acid-base pollution.

3.2. Physical method

Physical degradation are mainly based on direct heating, radiation, and ultrasonic treatment. It is difficult to produce oligosaccharides by one single physical degradation method, and the minimum molecular weight of the resulting products is about 50 kDa, so it is usually combined with other degradation methods (Yanaki *et al*. 2010). The direct heating method is *via* heating aqueous solution or

the dry powder of alginate to prepare the oligosaccharide by breaking the glycosidic bonds (often at the position of connecting M and G).

Radiation degradation does not require any additive, and is characterized by low cost, no chemical pollution, easy control and high product quality. When the alginates (solid or solution) are treated with γ-ray generated by Co60, the AOS with a molecular weight distribution from 1 to 10 kDa can be obtained according to the optimal radiation intensity (Nagasawa *et al*. 2000). Alternatively, using microwaves is also very convenient. Hu *et al.* (2013) obtained a mixture of oligomeric guluronic acid, which was generated by irradiation of microwaves at 130°C and 1 600 W for 15 min. The oligomeric guluronic acid has a polymerization degree from 2 to 8 as determined by electrospray ionization mass spectrometry and nuclear magnetic resonance analysis.

Overall, physical methods have the characteristics of simple operation, no chemical pollution and low cost, as there is no requirement of chemical reagents such as irritating acids and bases. However, physical methods could produce products with low purity and uneven degradation rates, and consequently the polymerization degree may exceed the range of AOS, ranging from 1 to 10 or higher.

3.3. Enzymatic method

Enzymatic degradation is to degrade the alginate by β-(1,4)-elimination reaction, to produce C4, five double bonds at the non-reducing end (Zhu and Yin 2015). The alginates are degraded by lyase under the specific reaction conditions, which is the unique property of the enzymatic method relative to the chemical or physical methods (Smidsrød *et al*. 1965).

Alginate lyase of enzymatic method is mainly derived from marine bacteria, fungi, algae, molluscs and soil microorganisms (Suzuki *et al*. 2006). By the year of 2000, more than fifty alginate lyases have been discovered, of which about twenty have been successfully cloned (Wong *et al*. 2000). They can be divided into three categories according to its degradation of alginate blocks: polyM enzyme specifically cleaves M segment, polyG enzyme specifically cleaves G segment, and the dual function poly MG enzyme can lyse both M and G segments (Osawa *et al*. 2005). In one study, one brown algae degrading enzyme was isolated and purified from *Vibro* sp. 510 (Zhang *et al*. 2004). After being hydrolyzed for 24 h followed by ethanol precipitation, seven different AOS with polymerization degree of 2–5 were obtained.

Alginate lyase is abundant innature, but its industrialization often meets with difficulty. The reason is that the cost of enzyme separation and purification is very high, and it is difficult to get separated and purified from the digestive gland of mollusks (Zhu and Yin 2015; Zhu B *et al*. 2016). Furthermore, it is difficult to separate and purify the highly specific enzyme product from the fermentation broth of marine bacteria (Horn and Østgaard 2001). However, enzymatic methods have the characteristics of no chemical pollution, high catalytic efficiency, mild reaction conditions and strong specificity. For example, Svanem *et al*. (2001) used the AlgE7 lyase to degrade M-rich alginates and a relatively G-rich alginates from the brown algae *Macrocystis pyrifera* most effectively, producing oligomers of 4 to 7 U. Therefore, the enzymatic method is the most effective and most promising method for the preparation of AOS. This also means that solving the insufficient alginate lyase industry by reducing production costs and improving the purity of enzymes, is the key to prepare AOS with the specific polymerization degree and high purity.

4. Biological activities of AOS

In recent years, AOS were gradually found to exhibit many important biological activities such as anti-oxidation, stimulating immunity, anti-bacterial and anti-tumor effects (Yoshiko *et al*. 2003; Hu *et al*. 2005; Jacobsen *et al*. 2019). AOS can promote the growth of *bifidobacteria* (Akiyama *et al*. 1992) and stimulate the growth of human keratinocytes and endothelial cells and promote the growth of plant roots (Tomoda *et al*. 1994; Kawada *et al*. 1997, 1999; Iwasaki and Matsubara 2000; Zhang *et al*. 2014).

4.1. Anti-oxidant effect

Due to obvious anti-oxidant effect, AOS are used as a new kind of feed additive in livestock and poultry, which can effectively improve the anti-oxidant capacity of farm animals (Jacobsen *et al*. 2019). Studies have shown that AOS can prevent lipid oxidation of skin care lotions and scavenge hydroxyl radicals and superoxide anion radicals (Zhao *et al*. 2012). Falkeborg *et al*. (2014) reported that AOS can completely (100%) inhibit lipid oxidation in skin care lotions, superior to vitamin C (89%), and exhibit an excellent scavenging activity to 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), hydroxyl radicals and superoxide anion; this study also revealed that the free radical scavenging ability of AOS is mainly derived from the conjugated alkenoic acid structure formed during the enzymatic degradation of alginate (Falkeborg *et al*. 2014). The lipid peroxide content can also be significantly reduced by increasing the activities of enzymes such as superoxide dismutase and peroxidase. The anti-oxidant mechanism of AOS is related to oxygen radical decomposition and consumption for the purpose of

free radicals disposal. However, some studies presented different results in support of different viewpoints. For example, Wang *et al*. (2007) found that AOS had no effect on the lipid oxidation of the emulsion, only cleared the hydroxyl radicals and could not scavenge the superoxide anion radicals. In addition, the study found that the activity of scavenging free radicals of AOS is dose-dependent, and the molecular weight and M/G ratio are important factors affecting the anti-oxidant activity of AOS. Moreover, the free radical elimination activity has a negative correlation with their molecular weight (Sen 2011; Ueno *et al*. 2012). In short, as an excellent natural anti-oxidant, AOS have great potentials of application in the field of human health and feed additives in livestock and poultry.

4.2. Immunomodulatory effect

In vitro and *in vivo* studies have shown that AOS have the function of enhancing immune activities, regulating immune system through multiple ways, including the regulation of various cytokines and complement secretions. *In vitro* studies have found that AOS can increase the secretion of IFN-γ and IL-12, and reduce the level of IL-4, thereby promoting the proliferation of Th1 cells and reducing the production of IgE and allergic reaction (Yoshida *et al*. 2004; Uno *et al*. 2006). AOS G could readily activate macrophages and stimulate TLR4/Akt/NF-κB, TLR4/Akt/mTOR and MAPK signaling pathways to exert its immunological activity (Fang *et al*. 2017). Uno *et al*. (2006) found that oral administration of AOS could inhibit the production of IgE and prevent allergic reactionsin mice. Yamamoto *et al*. (2007b) also compared the effects of AOS and alginates on the immune function in mice, and found that intraperitoneal injection of 700 mg kg^{-1} AOS could increase the level of twenty cytokines in serum, while little such observation was found for alginate.

The immunomodulatory activity of AOS is affected by multi-factors: degree of polymerization, purity, M/G ratio and MG sequence. Xu *et al*. (2014) found that unsaturated guluronic acid prepared by enzymatic degradation could induce nitric oxide (NO) production and increase the expression of inducible nitric oxide synthase (iNOS) in mouse macrophages RAW264.7, in a dose- and time-dependent way, but AOS prepared in other ways did not have these effects. It suggests that structural features such as unsaturated terminal structure, molecular weight and M/G ratio play important roles in AOS-activating macrophages. Yamamoto *et al*. (2007a) pointed out that PM tended to be more potent than PG in the activities of cytokine induction, indicating that molecular conformation and size are important in immunomodulatory activity of AOS. Otterlei *et al*. (1991) found that M is the main factor influencing AOS-activating macrophages. AOS can activate the innate immunity by recognizing macrophage Toll-like receptors. Further research of AOS is warranted about immune regulation signaling. Otterlei *et al*. (1993) pointed out that the pattern of the induction of TNF-α by AOS is similar to that of microbial Lipopolysaccharide (LPS), which can bind to the CD14 receptor on the surface of monocytes and requires the involvement of serum and LPS-binding proteins to induce secretion of TNF-α. Presumably AOS and LPS have very similar stimulation pattern for monocytes.

4.3. Anti-bacterial and anti-tumor activities

AOS were reported with robust anti-bacterial effects. Hu *et al*. (2005) evaluated the *in vitro* anti-bacterial activity of AOS with different molecular weights on 19 bacterial strains. AOS with molecular weight of 4.235 kDa showed the broadest and the strongest anti-bacterial activity. The minimum inhibitory concentration (MIC) of AOS against *Escherichia coli*, *Salmonella paratyphi* A, *Staphylococcus aureus* and *Bacillus subtilis* were 0.312, 0.225, 0.016 and 0.325 μg mL^{-1}, respectively. AOS also had a strong effect in inhibiting the growth of yeasts *Candida* (*n*=11) and molds *Aspergillus* (*n*=3), with a significant dose-dependent effect (Tøndervik *et al*. 2014). In addition, AOS exerted bacteriostatic action by destroying biofilms of two Gram-negative bacteria, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (Wright 2013). Tøndervik *et al*. (2014) reported that AOS significantly destroyed the biofilm formation of *Candida* and *Aspergillus*. Moreover, AOS enhanced the membrane-damage effect toward fungal organisms combined with anti-biotics such as miconazole, fluconazole and terbinafine (Tøndervik *et al*. 2014). In addition, AOS also has a prebiotic effect. Akiyama *et al*. (1992) demonstrated that the addition of AOS to the culture medium significantly promoted the growth of five *bifidobacteria*, and their effect was even better than that of lactulose and galactooligosaccharides. By feeding mice with AOS, the number of *bifidobacteria* and *lactobacilli* in the mice feces greatly increased, and the number of *enterococci* and *enterobacteria* significantly decreased, which confirmed that AOS could regulate intestinal bacteria and bacteria metabolites (Wang *et al*. 2006).

Historically, AOS were found to have anti-tumor activity. Chen *et al*. (2017) found that AOS could reduce the development of osteosarcoma. After two years of treatment, the average tumor volume of patients in the AOS intake group was significantly lower than that of the control group, and the local recurrence rate was greatly lower than that of the control group (44.9% *vs*. 68.7%). Moreover, AOS had an inhibitory effect on proliferation of human leukemia U-937

cells and could produce cytotoxins in human monocytes (Yoshiko *et al*. 2003). Hu *et al*. (2004) reported that two kinds of AOS with different molecular weights showed no direct cytotoxicity to tsFT210 cells, subsequently, AOS with molecular weight of 3.798 kDa and sulfation degree of 1.3 having anti-tumor activity was confirmed in mice, although the effect was optimized and the inhibition rate of solid sarcoma 180 could reach 70.4% when adding 100 mg kg^{-1} AOS.

4.4. Growth promoting activity

AOS have an activity of promoting human cell growth. Kawada *et al*. (1997) found that AOS as a cofactor of epithelial growth factor (EGF) could promote the growth of human keratinocytes, and its promoting activity was quite equivalent to bovine pituitary extract (common complement in keratinocyte culture) activity. When vascular endothelial factor 165 (VEGF165) was added, as low as 5 μg mL^{-1} concentrations of AOS could stimulate the growth and migration of human umbilical vein endothelial cells (Kawada *et al*. 1999).

The growth promoting effect of AOS on plant roots was also significant. The 2–8 sugar mixtures of AOS could significantly promote the growth of lettuce roots. The findings showed that AOS with 3, 4, 5 and 6 polymerization degrees all had promoting effect (Iwasaki and Matsubara 2000). The AOS mixture also has promoting effect on the growth of barley roots. Natsume *et al*. (1994) found that the 3- and 4-degree polymerization of AOS significantly promoted the growth of barley roots. Tomoda *et al*. (1994) found that the roots began to gradually elongate within 2–4 h after the contact of barley radicle with AOS. The explanation was that AOS could induce the production of NO in wheat roots by up-regulating the expression of nitrate reductase gene and promoting enzyme activity, of which NO played an important role in plant root growth (Zhu W H *et al*. 2016).

In summary, AOS play an important role in anti-oxidation, regulating immunity, anti-bacterial and anti-tumor activities and growth promoting. Their molecular weight, M/G ratio, MG sequence and the conformation of the entire molecule are all important factors for AOS biological functions.

5. Applications of AOS in livestock and poultry

Numerous experiments and reports have confirmed that AOS have various biological activities. In consideration of their different biological activities, some research treated AOS as feed additives, reported that AOS have good efficacies in promoting growth and improving health of livestock and poultry.

5.1. Applications of AOS in pigs

Studies in weaned piglets showed that AOS as feed additives could promote growth, improve nutrient digestibility, enhance immunity and anti-oxidant capacity. Zhu W H *et al*. (2016) conducted a 28-d experiment using 180 crossbred Duroc×(Landrace×Yorkshire) piglets weighing (9.19±1.47) kg and weaned at 35 days of age. They fed the piglets with corn-soybean meal based diets supplemented with 0, 3, 4, 5 or 6 g kg^{-1} AOS PM (self-made) with an average molecular weight of ~10 kDa, and each treatment was replicated 6 times with 6 pigs per replicate. The results showed that AOS PM as feed additives could promote piglet growth (increase the average daily gain and weight gain/consumption ratio) and improve the anti-oxidant capacity of piglets (serum GSH content was increased).

Another research group of Wan *et al*. (2017, 2018a, b, c) have done a series of trials to investigate the biological functions of AOS on weaned piglets. In 2017, they evaluated the effects of AOS on the growth performance, anti-oxidant capacity, serum hormone levels and intestinal digestion-absorption function with two trials. In trial 1, 200 weaned pigs (Duroc×Landrace×Yorkshire, weaned at 21 d), with an initial body weight of (6.17±0.16) kg, were allotted to 4 groups with 5 replicate pens per treatment and 10 pigs per pen, and fed diets containing 0, 50, 100 or 200 mg kg^{-1} AOS with an average molecular weight of <1 000 Da. In trial 2, 24 weaned pigs (Duroc×Landrace×Yorkshire, weaned at 21 d), with an initial body weight of ((6.21±0.09) kg), were allotted to a control group (fed with a basal diet) or an AOS group (basal diet containing 100 mg kg^{-1} AOS). The experimental results revealed that 100 or 200 mg kg^{-1} AOS supplementation significantly increased the average daily body weight gain of the pigs compared to the control group, and 100 mg kg^{-1} was confirmed as the minimum effective level in weanling piglets; effectively promoted anti-oxidant defense properties by enhancing serum catalase activity and GSH content; improved intestinal enzyme activity and digestibility of nutrients. Through this experiment, the group of Wan *et al*. (2017) regarded the gut tissues as the main target of AOS.

Then they focused on the effects of AOS on piglets' intestinal barrier function. In the trial, 24 weaned pigs (Duroc×Landrace×Yorkshire, weaned at 21 d), with an initial body weight of (6.21±0.09) kg, were allotted to a control group (fed with a basal diet) or an AOS group (basal diet containing 100 mg kg^{-1} AOS with an average molecular weight <1 000 Da). The results showed that AOS could enhance intestinal integrity through increasing intestinal closure protein abundance and inhibiting the secretion of inflammatory factors. They pointed out that AOS inhibited the production of pro-inflammatory cytokines and protected

the cell integrity of the intestinal wall by inhibiting TLR4/NF-κB and NOD1/NF-κB signaling pathways (Wan *et al*. 2018b).

Further research was conducted to evaluate whether AOS supplementation could attenuate enterotoxigenic *Escherichia coli* (ETEC)-induced intestinal mucosal injury in weaned pigs. Twenty-four weaned pigs (Duroc×Landrace×Yorkshire, weaned at 21 d), with an initial body weight of (6.58±0.12) kg, were randomly assigned to 3 treatments: (1) non-challenged control; (2) ETEC-challenged control; and (3) ETEC challenge+AOS treatment (100 mg kg^{-1}), the AOS with an average molecular weight <1 000 Da. The research found that AOS supplementation could attenuate ETEC-induced intestinal mucosal injury in weaned pigs. They deduced that the protective effects may be associated with the restrained enterocyte death, by reducing both mitochondria-dependent and TNFR1-dependent apoptosis and the accelerated enterocyte proliferation, *via* enhancing the cyclin E-CDK2 complex formation (Wan *et al*. 2018a).

In addition, Wan *et al*. (2018c) conducted another trial to investigate the AOS-mediated changes in the growth performance of weaned pigs. Twenty-four pigs (Duroc×Landrace×Yorkshire), weaned at 21 d and with an average body weight (BW) of (6.21±0.09) kg, were assigned to 2 treatments: a control group (CON), in which pigs were fed a basal diet, and an AOS group, in which pigs were fed a basal diet supplemented with 100 mg kg^{-1} AOS, with 12 replicates per treatment. The result showed that 100 mg kg^{-1} AOS supplementation ameliorated the intestinal morphology and barrier function (increased villus height, content of sIgA and goblet cell counts) and prevented the intestinal epithelial cell apoptosis, which was possibly elicited by reducing mitochondria-dependent apoptosis in intestinal epithelial cell, promoting the cell cycle from G0/G1 to S phase, and increasing jejunal epithelial cell proliferation (Wan *et al*. 2018c).

Similarly, Zhang *et al*. (2018) studied the effects of AOS. A total of 720 Duroc×Landrace×Yorkshire weaning piglets, (25±1) d, were randomly allocated into the 4 groups which were fed with the basal diet containing 0, 20, 40 and 80 mg kg^{-1} AOS, with 6 replicates of 30 pigs. They found that dietary supplementation of AOS significantly increased the daily weight gain and feed intake of piglets compared with the control group, the apparent ileal digestibility of crude protein, crude ash, crude fat, total energy, and the activity of maltase and sucrase were increased in the duodenum and jejunum. They identified AOS as a potent compound that reversed weaning-induced oxidative stress and intestinal digestive disorders in pigs.

The above results show that AOS have positive effects on the growth, immunity and anti-oxidant capacity of pigs. However, the ideal dose of AOS in different growing periods of different animals should be further investigated.

5.2. Applications of AOS in poultry

The effects of AOS on the performance and immune system of poultry have been intensively investigated. Zhu *et al* (2015) assessed the effects of AOS PM (self-made) on the performance, anti-oxidant capacity, immune status and cecal fermentation profile of broiler chickens. A total of 540 one-d-old male Arbor Acres broilers, with an average BW (43.77±1.29) g, were randomly divided into 5 treatments with 6 replicates of 18 chicks and fed a corn and soybean meal (SBM)-based diet supplemented with 0, 1, 2, 3, or 4 g kg^{-1} AOS PM of which the average molecular weight was ~10 kDa. They found that supplementation with AOS PM significantly increased the feed intake and growth performance of chickens. From the perspective of anti-oxidant capacity and immune status, they showed that AOS PM increased plasma glutathione (GSH) levels, serum IgM levels and thymus index while reduced malondialdehyde (MDA) content in thoracic tissue. Besides, AOS PM increased the number of beneficial lactic acid bacteria in the cecal of the broiler chickens, reduced the number of harmful *E. coli*, and increased the content of acetic acid, lactic acid and total volatile fatty acids in the cecum (Zhu *et al*. 2015).

In another study, the effects of AOS from brown algae on the growth performance, cecal microbiota, *Salmonella* translocation to internal organs, and mucosal immune responses of broiler chickens challenged with *Salmonella enterica* serovar Enteritidis were investigated. A total of 360 one-d-old male Arbor Acres broiler chicks were assigned to 6 treatments consisting of 5 replicates of 12 chicks each in a 2×3 factorial arrangement of treatments. There were 3 feed treatments with supplementation of 0 (control), 0.04, or 0.2% AOS (purity ≥90%) in the diets of non-challenged birds and birds challenged with *Salmonella* enteritidis. The results showed that the body weight loss and mortality resulting from *Salmonella* infection were mitigated by the addition of AOS. Supplementation of AOS at 0.2% significantly reduced *Salmonella* colonization and increased the number of lactic acid bacteria in the cecum of chickens. In addition, cecal *Salmonella* enteritidis-specific IgA production was increased by AOS at 0.2% at 5 d post challenge compared with the other treatments (Yan *et al*. 2011). These findings suggest that dietary AOS can decrease *Salmonella* colonization and improve intestinal barrier function and performance of chickens challenged with *Salmonella enteritidis,* and the supplementation of AOS at 0.2% was the most effective concentration.

5.3. Applications of AOS in ruminants

At the present, no researches on the AOS application in dairy cows, beef cattle, sheep and goats have been reported yet. This may be due to the insufficient industrialization of alginate lyase (a major bottleneck), which is concomitant with high production cost in preparation of AOS and hence relatively few practical applications in large domestic animals. Next, in response to the problems of ruminant health issues and cow mastitis, etc., studies can be carried out to evaluate the role of AOS in improving production performance and rumen health, as well as preventing or alleviating mastitis, according to the exemplary evidence from pigs and broilers.

Despite of the fact that more research is warranted for the application of AOS, especially in dairy cows, beef cattle, sheep and goats, it seems that AOS has great potentials as a new green feed additive in livestock and poultry husbandry.

6. Conclusion

As the compulsory withdrawal of anti-biotics from Chinese feed market in 2020 is imminent, it is urgent to develop new, safe and efficient functional feed additives and anti-biotics alternatives to ensure the healthy development of livestock and poultry. With many biological activities such as anti-oxidation, immunomodulation and anti-bacterial effects, AOS might be excellent potential anti-biotic alternatives. The development and utilization of AOS as feed additives will definitely contribute to the development of livestock and poultry production. However, there is still much work to be done, such as breaking through the problem of insufficient alginate lyase industry, reducing production costs and improving the purity of enzymes. Because of self-made AOS products used in previous studies, the composition and structure of AOS products were quite different, and the amount of tested AOS additives varied greatly. Moreover, techniques of AOS for application in feed additives as alternatives of anti-biotics are needed to develop through many pilot animal trials. Therefore further researches are needed to determine the optimal structural composition and the optimal supplementation of the AOS in improving the performance of livestock and poultry, and to provide a solid theoretical basis for the wider applications of AOS in the future.

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