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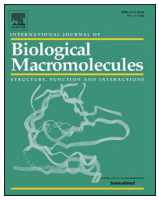

Review

The role of alginate oligosaccharide on boar semen quality: A research review

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ABSTRACT

Alginate is the general term of a polysaccharide which is widely used in the area of pharmaceutics and the food industry and is known for its unique biological activities. However, due to the low water solubility and large viscosity of alginate, its development and utilization in the agricultural field are limited. Alginate oligosaccharide (AOS) is a degradable product derived from alginate and has attracted much attention in recent years because of its specific characteristics such as a low molecular weight, high water solubility, and non-toxicity.

Boar semen quality, which is affected by various factors, is an important indicator for measuring reproductive performance of boars. With the development of artificial insemination technology, high quality semen has been more and more important. Therefore, increasing semen quality is an important means to improve the reproductive performance in swine industry.

In this research review, we used the PubMed database and Google Scholar and web of science to search for relevant literature on the topic of AOS in relation to boar semen quality. Key words used were alginate oligosaccharide, boars, semen quality, microbiota and metabolites. The purpose of this review article was to describe the current knowledge on the relationship between AOS and boar semen quality, and provide an overview of solutions for the decline in the boar semen quality in specific conditions. Based on the existing literature, it is evident that AOS can be used as a new type of food additive. This review paper provides a theoretical basis for the production of high-quality boar sperm and, suggests that, in the future, AOS can even aid in treating human infertility.

1. Introduction

Nowadays, there are two main ways to obtain substances with biological activity. On the one hand, they can be extracted from natural plants [1], on the other hand, they can be obtained by artificial synthesis [2,3]. Alginate is a natural polysaccharide polymer that exists in the wall of brown algae cells. It is composed of monomers containing carboxyl groups. Alginate has been widely used in the pharmaceutical and food industry due to its unique physicochemical properties and beneficial health effects. Its molecular formula is $(C_6H_7O_6Na)n$ [4]. Alginate connects α-l-Guluronic (G) acid and β-d-Mannuronic acid (M) monomers through an 1,4-glycoside bond forming a long chain polymer. Therefore, there are three different kinds of combinations such as Polymannuronate (PM), Polyguluronate (PG) and Heteropolymer (polyMG) [5] as shown in Fig. 1. With the continuous deepening research on alginate, it is gradually considered to be a macromolecular compound with anti-coagulation and anti-viral functions. However, due to its large molecular weight and viscosity, it is difficult to cross the cell membrane and multiple biological barriers, thereby limiting its utilization and development [6]. Alginate oligosaccharide (AOS) is a decomposition product of alginate and has received increasing attention due to its low molecular weight, high water solubility, safety, and non-toxicity [7]. AOS has a variety of biological activities, including immune regulation [8], and it has anti-inflammatory [9], antioxidant [4] and even anticancer properties [10]. According to publication and patent statistics, the number of AOS-related outputs have grown continuously during the

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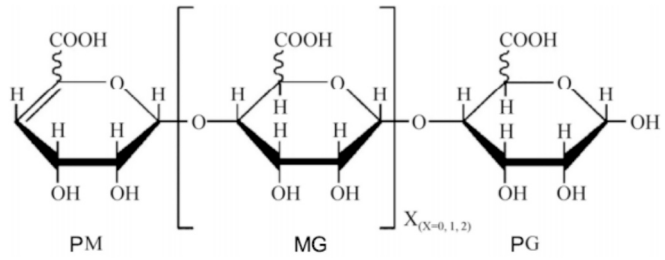

Fig. 1. Molecular structure of alginate. PM means polymannuronate, PG means polyguluronate, MG means heteropolymer,

half century from 1968 to 2023 (as shown by the search of "Alginate oligosaccharide" via Web of Science). Prior to year 2000, the number of outputs was 96, but from 2000 to 2009 it increased to 263, and has surged to 836 in the last 10 years.Therefore, AOS has received extensive attention, especially in the past decade, with the number of articles published on AOS-related research increasing year by year (Fig. 2A). Statistical analysis of Web of Science showed that basic research on AOS and its applications is mainly concentrated in related fields such as biochemistry and molecular biology, chemistry, biotechnology and applied microbiology, food science and technology, microbiology, pharmacology pharmacy and agronomy (Fig. 2B). AOS is extremely widely used in pharmaceutical research, as well as in the functional food industry and the agricultural field.

In modern swine breeding, the application of artificial insemination technology has become more and more common. Therefore, the fertility of boars will not only directly affect the economic benefits of the commercial boar studs, but also indirectly affect the reproductive performance of sows. The fertility of boars can be evaluated with indicators such as sexual libido, semen quality, in vitro fertilization, and sow pregnancy rate [11,12]. Among them, semen quality is the most important index to evaluate boar fertility [13,14]. Generally, we use indicators such as sperm volume, sperm density, sperm motility, and sperm morphology to evaluate semen quality [15]. The amount of sperm volume and sperm density determines the total sperm of ejaculation, which mainly reflects the spermatogenesis of testicles [16]. It will affect the number of mating sows and pregnancy rate during artificial insemination. Sperm motility and sperm morphology are two core indicators that jointly determine the semen quality and affect the pregnancy rate of sows [17]. In production, when the semen stores a period of time at room temperature, the sperm motility becomes lower and the abnormal sperm rate is increased, which is the main reason for the decline in boar fertility [18]. Although the number of input sperm can be increased during artificial insemination to solve the problem of decreased

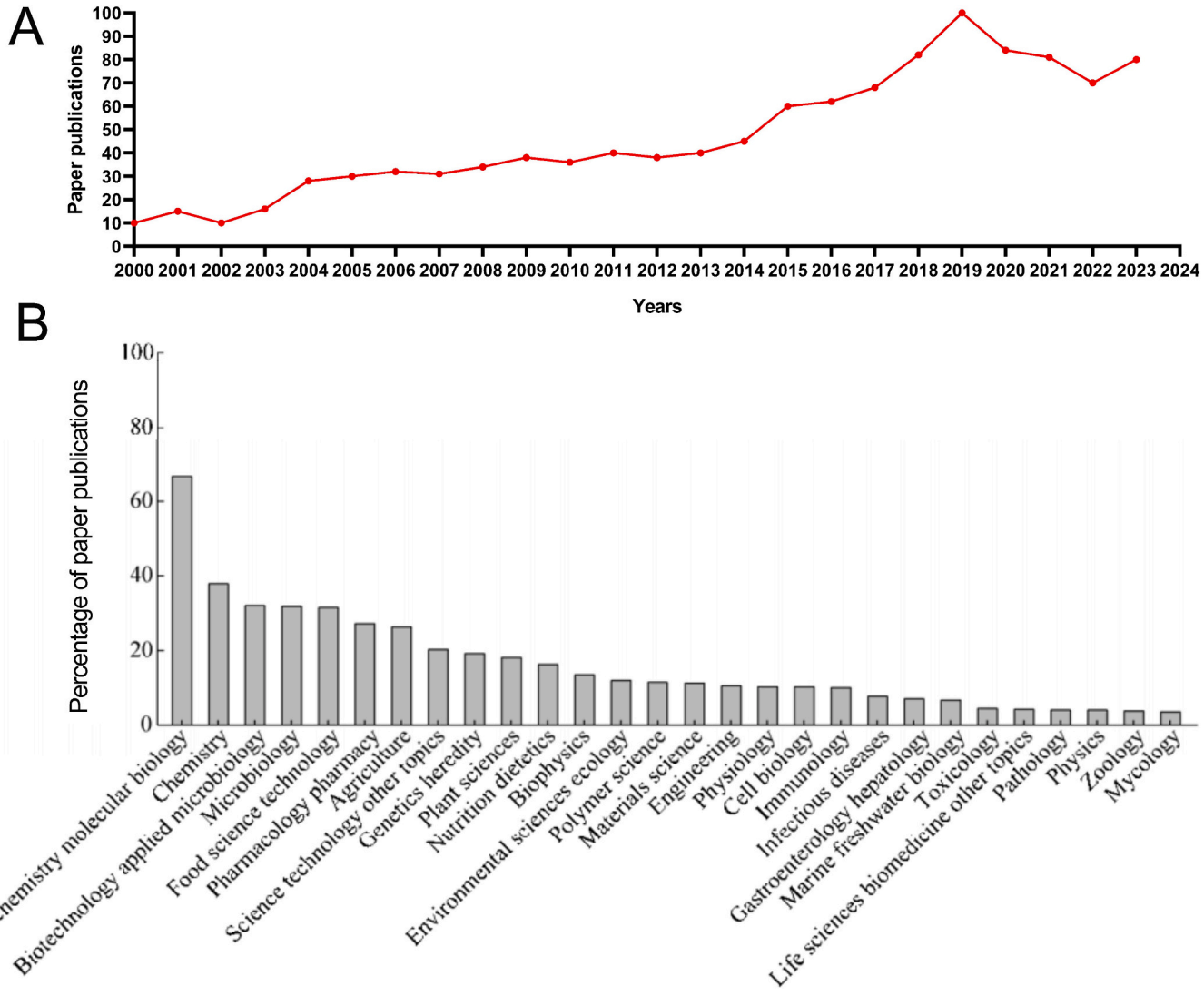

Fig. 2. An overview of AOS related articles published in the past 23 years. (A) The number of annual publication of AOS during 2000–2023. (B) Distribution of research areas associated with AOS-related publications.

pregnancy rate, the primary goal of improving semen quality is still to improve sperm motility [19]. This is also the research hotspot of boar fertility through nutritional regulation technology. The semen quality is closely related to the spermatogenesis, which is affected by the several factors such as genetics, environment and nutritional management [20]. This article reviews the biological functions of AOS and the factors that affect the semen quality, aiming to find the mechanism of how AOS can regulate the boar semen quality so as to provide a theoretical basis for improving the swine reproductive industry.

2. The biological activities of AOS

Alginate is a biopolymer used extensively in the food, pharmaceutical, and chemical industries. Alginate oligosaccharide (AOS) derived from alginate exhibits superior biological activities and therapeutic potential. Alginate lyases with characteristic substrate specificity can facilitate the production of a broad array of AOS with precise structure and functionality. As a natural plant extract, AOS exhibits multiple bioactivities including anti-oxidant, immune regulation, anti-bacterial and anti-inflammatory activities. In this section, we mainly talk about the main functions of AOS, as shown in Fig. 3.

2.1. Immune regulation properties of AOS

Natural polysaccharides and oligosaccharides have the potential to be immune regulators. One of the key functions of AOS in immune regulation is the ability to induce cytokine expression [21]. It is found that AOS can increase the concentration of IL6, IL10, and IgA in the serum of weaning piglets, as well as the level of IgA in the small intestine [22]. This indicates that AOS has the potential to elevate the levels of immunoglobulins and cytokines, thereby enhancing the immune function of weaning piglets. In a study on macrophages, alginate was seen to induce the secretion of TNF-α, and AOS obtained by alginate cracking augmented the induction activity of TNF-α compared to intact alginate [23]. Using different methods to crack alginate, the obtained AOS has different chemical groups, and AOS-induced cytokine secretion is closely related to its structure. At present, more and more studies are concerned about the relationship between the unsaturated terminal structure of AOS and its activity. AOS obtained by microorganisms to crack alginate contains more unsaturated double bonds on the terminal structure than AOS obtained by acid degradation methods. As unsaturated groups play a key role in the secretion of TNF-α in macrophages, the degradation method used is important [24]. In addition, AOS can increase the expression of induced nitric oxide (iNOS) synthase in macrophages, promoting the generation of nitric oxide (NO), and stimulating the generation of reactive oxygen species (ROS) and TNF-α [8].

2.2. Anti-inflammatory properties of AOS

Inflammation is an important defense mechanism for the body to resist pathogen invasion and it will activate inherent immune cells. However, inflammation will also appear in the process of chronic diseases, such as arthritis and diabetes [25]. AOS can improve the intestinal anti-inflammatory ability of weaning piglets by inhibiting the TLR4/NF-κB and NOD1/NF-κB signaling pathways [26]. The anti-inflammatory ability is also related to the preparation methods used for AOS production. AOS prepared through oxidation degradation has a stronger inflammatory inhibitory effect compared to AOS prepared by other methods. AOS extracted by oxidation degradation can inhibit LPS/β-amyloid induced neuritis, by promoting β-amyloid phagocytosis effects [27]. This is done by blocking NF-κB and MAPKs signaling pathways. Contrarily, AOS prepared by microorganisms has no anti-inflammatory effects [28]. The anti-inflammatory AOS enhances the function of small intestinal epithelial cells by increasing the proportion and number of various cell clusters in the small intestine such as goblet cells [29], thereby alleviating small intestinal mucositis induced by Busulfan. Furthermore, a study in IPEC-J2 cells has found that AOS can play a significant anti-inflammatory role through the mannose receptor signal pathway [30]. In addition, AOS is used as an anti-inflammatory drug in clinical trials. Studies have shown that AOS has immunosuppressive effects in various tested mice models used for arthritis, nephrotic syndrome, and glomerulonephritis [31]. Its anti-inflammatory activity is related to the regulation of the TLR4 signal pathway provoking inflammation [32–34].

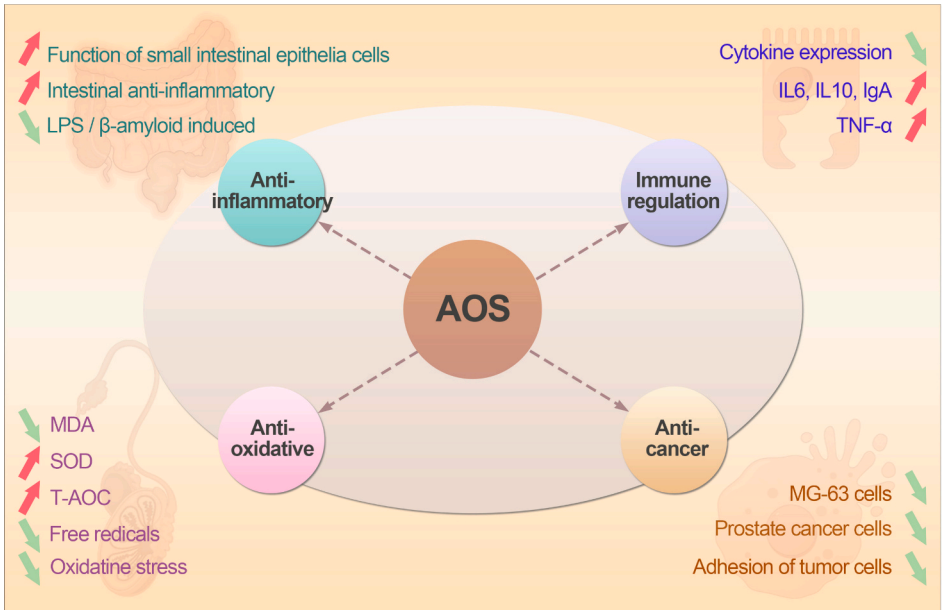

Fig. 3. The four main biological activities of AOS: immune regulation, antioxidation, anti-cancer function and anti-inflammatory properties. For the immune regulation, AOS influences cytokine expression. AOS improves the function of small intestinal epithelial cells and helps against intestinal inflammatory challenges such as those induced by LPS. AOS increases the antioxidant indicators SOD and T-AOC, and decreases MDA and ROS. AOS decreases the proliferation and migration of cancer cells and therefore achieves an anti-cancer ability. Red arrow show increase, green arrow show decrease.

2.3. Anti-oxidative properties of AOS

AOS acts as a very strong antioxidant by effectively removing free radicals. Since AOS prepared by microbial engineering (*Microbulbifer*) mainly consists of disaccharides and trisaccharides, it has the ability to remove free radicals (DPPH and Hydroxyl radical), thereby promoting restoration [35]. A Study by Fu et al. has shown that AOS prepared by enzyme degradation can have a stronger antioxidant activity than AOS prepared by other methods, the reason for that being its structure of more double bonds [36]. The antioxidant mechanism of AOS may be linked to a way in which hydrogen ions form a hydrogen bond with free radicals, forming a more stable product, and leading to the subsequent removal of free radicals [37]. In animals, AOS has the effect of resisting oxidative stress and preventing oxidative damage. A study of Tusi et al. has shown that in an oxidative stress model, caused by hydrogen peroxide, AOS can block H_2O_2-induced oxidative stress and caspase-dependent apoptotis in both the endoplasmic reticulum and mitochondria [38]. AOS can inhibit the expression of GP91 and 4-hydroxylceride, thereby reducing heart oxidative stress, increasing the survival rate of mice damaged by adriamycin [39]. In a study by Li et al., AOS could significantly reduce the level of hydrogen peroxide and MDA in mice, thereby reducing ROS production [40]. AOS was also found to improve serum levels of SOD, peroxide and total antioxidant capacity (T-AOC) in weaning piglets, and to reduce the content of MDA in the serum and small intestine [26]. In addition, adding AOS to a broiler diet increased the concentration of glutathione peroxidase in the serum, and reduced the content of MDA in the liver, thereby improving the antioxidant capacity in broilers [41]. To conclude, AOS can improve the antioxidant capacity in animals by modulating the enzymatic antioxidant system, thereby preventing lipid peroxidation of animal tissues.

2.4. Anti-tumor properties of AOS

AOS exhibits anti-tumor activity by inhibiting the proliferation and migration of tumor cells. A study by Han et al. has proven that AOS can weaken the proliferation, migration and invasion of human prostate cancer cells by inhibiting the Hippo/YAP/C-JUN signaling pathways [10]. AOS obtained by *Pseudoalteromonas,* used for alginate degradation, can enhance the defense mechanism against leukemia cells by raising cytokines synthesized by monocytes, a process that is inhibited by the TNF-α antibody [42]. Fifty mg/kg AOS can effectively inhibit the adhesion of tumor cells on the extracellular matrix, inhibit the infiltration of tumor cells in tissues, and reduce the accumulation of immune suppression cells and inflammatory cells in mice [43]. Therefore, the anti-tumoral and immune regulatory effects of AOS are inseparable. A study by Chen et al. has shown that AOS can inhibit the growth of MG-63 cells in human skeletal cells [44]. AOS selectively induces TNF in tumor tissues, leading to a localized treatment effect on liver cancer in mice [45]. In addition, 10 mg AOS per day for two consecutive years has been found to improve the antioxidant and anti-inflammatory ability of patients with osteosarcoma by reducing the tumor volume and the recurrence rate [46]. Therefore, to conclude, AOS plays a significant role as anti-inflammatory and antioxidative agent, while also showing anti-tumoral effects.

3. Evaluation parameters and detection methods of boar semen quality

Boar semen quality is directly related to it fertilization ability [47]. At present, semen quality parameters include sperm motility, sperm concentration, sperm vitality and abnormal sperm rate [48]. These indicators will directly or indirectly reflect the quality of sperm. In the evaluation method of boar semen, the traditional method, called hematimetry, using a hemocytometer, determines the sperm number of sperm with a forward movement. Another method is the Giemsa dyeing method to determine the abnormal sperm rate. While these methods are accurate, they consume considerable time and labor [49]. The computer-assisted sperm analysis (CASA) system can also provide accurate results and is less time and labor-intensive [50]. Therefore, the CASA system has obvious advantages for artificial insemination (AI) in animal husbandry, the semen quality parameters that are suitable for AI is shown in Table 1.

3.1. Sperm motility

Sperm motility refers to the ability of sperm to move forward. A study by Mahajan et al. found that sperm motility is an important evaluation parameter for semen analysis. Its percentage can directly affect the quality of semen and its fertilization ability [51]. Therefore, using the CASA system, conducting a quantitative analysis of sperm motility, can lead to a more accurate assessment. In pig production, sperm motility is generally higher than 75 %, and meets the standards for AI [52].

3.2. Sperm density

Sperm concentration refers to the total number of sperm per ml semen. Sperm concentration is an important indicator of semen quality, as it will directly affect the dilution times in the AI process, thereby affecting the pregnancy rate of sows [53]. At present, sperm concentration evaluation methods are diverse. As such there is the absorption spectrophotometry method, the hematimetry method, as well as the aforementioned computer assisted CASA method [54]. Hematimetry is the most accurate method for evaluating sperm concentration. However, in pig production, CASA analysis can quickly detect sperm concentration and saves a lot of time. Generally, the sperm concentration should reach 10^8 per ml for it to meet the requirements for AI [55].

3.3. Sperm vitality

Sperm vitality refers to the percentage of total sperm that moves forward in the semen. Sperm vitality is closely related to the conception rate [56]. The use of the naked eye and calculators to evaluate the percentage of sperm with a straight line motion is difficult and often susceptible to the influence of human factors, which makes the results highly variable. In contrast, the results of CASA analyses are more accurate. In pig production, the sperm vitality requires to be 85 % to perform AI.

3.4. Sperm malformation rate

Abnormal sperm rate refers to the percentage of deformed sperm in the semen. Abnormal sperm rate is one of the important indicators to establish semen quality. An excessive malformation rate will significantly reduce the fertilization capacity of sperm [57]. In the process of fertilization, if the abnormal sperm rate is higher than 18 %, the ability of fertilization will be significantly reduced. Sperm malformation can occur in the whole sperm cell, and commonly appears in the sperm head,

Table 1
The boar semen quality that meets artificial insemination.

Semen quality	Method	Standard for AI	References
Sperm Motility	CASA	More than 75 %	Waberski et al., 2019; Mahajan et al., 2015
Sperm Density	CASA	More than 6×10^8/ml	Lesani et al., 2020; Morrell, 2019; Chanapiwat et al., 2014
Sperm Vitality	CASA	Straight line movement more than 85 %	Bibi et al., 2022
Abnormal Sperm Rate	CASA	Less than 20 %	Li et al., 2023; Fedder et al., 2007

mid-body or sperm tail. Sperm head malformations mainly include a knobbed acrosome [58] and pyriform heads [59]; mid-body abnormalities mainly include segmental aplasia [60] and distal reflex [61]; tail abnormalities mainly include an abaxial tail [62]. In the process of AI, the malformation rate detected by the CASA system should be less than 20 % for it to meet the standard requirements [63].

4. The role of AOS on boar semen quality

The way AOS regulates boar semen quality is mainly divided into three aspects. Firstly, AOS can improve sperm metabolites such as retinol that can act as antioxidant. Retinol can reduce ROS in the epididymis where the sperm stored. Secondly, AOS can improve sperm proteins such as protein kinase A (PKA), which is usually related to spermatogenesis and acrosome integrity. Thirdly, AOS can improve the composition of intestinal microorganisms. On one hand, AOS increases the relative abundance of beneficial bacteria such as *Butyricicoccus* which can produce butyric acid that is beneficial to spermatogenesis, on the other hand, AOS reduces the relative abundance of harmful bacteria such as *Prevotella*, that has a negative correlation with sperm motility and sperm concentration. The effects of AOS on the semen quality of boars are shown in Fig. 4.

4.1. AOS improves sperm metabolites to increase semen quality

There are many nutrients in semen that can directly or indirectly regulate semen quality [64]. A Study by Han et al. has shown that adding 10 mg/kg AOS to the basal diet of boars with similar age (33 months old) and body weight (300 kg) can significantly improve the sperm metabolome [65]. Among the metabolites that were enriched are antioxidant substances such as retinol, unsaturated acids such as DHA and EPA, and amino acids such as lysine. A study by Keys and Zimmerman has shown that retinol is a very strong antioxidant able to reduce reactive oxygen species (ROS) [66]. As we all know, mature sperm will temporarily store in epididymis [67]. In the epididymis, the mitochondria in the sperm generate a large amount of ROS through the oxidation reactions of the respiratory chain [68]. These ROS mainly include oxygen free radicals and hydroxyl radicals. Retinol has been found to reduce ROS in the epididymis and thus improve semen quality [69]. Unsaturated fatty acids such as DHA and EPA have also been shown to significantly improve semen quality in human. A study by Hosseini et al. using meta-analysis has shown that supplementing infertile men with omega-3 fatty acids resulted in a significant improvement in sperm motility and sperm concentration [70]. Another study in which boar spermatozoa were used as a model to investigate the impact of different glucose concentrations, shows a positive impact on spermatozoa motility, mitochondrial activity, and acrosome integrity at physiological temperature (37 °C) as well as during refrigeration (17 °C) by adding lysine [71]. These aforementioned sperm metabolites are significantly positively correlated with sperm motility and negatively correlated with abnormal sperm rate. This indicates that AOS can improve semen quality by improving sperm metabolites. Also, in a study with aging boars (65 months old Duroc boars with 300 kg body weight), AOS significantly improved the sperm metabolome [72]. Among them, the level of the antioxidant succinic acid significantly increased, while the level of dibutyl phthalate, which is negatively related to spermatogenesis, significantly reduced [73]. As a result, the service lifespan of Duroc aging boars could be extended through nutritional regulation. In addition, AOS can also improve the sperm metabolome to relieve the decline in semen quality caused by heat stress in boars [74], as could be seen by both the increased sperm motility and concentration after AOS supplementation. Moreover, in a study with mice, it was found that AOS repaired testicular damage caused by busulfan, and increased the sperm metabolites such as retinol, thereby increasing the sperm motility [75,76]. Therefore, AOS regulates the semen quality of boars through improving the sperm metabolome.

4.2. AOS improves sperm proteins to increase semen quality

Proteins play a vital role in the spermatogenesis, and the addition of AOS in the feed can increase the level of some specific proteins in the semen, thereby contributing to the improvement of semen quality. The proteins referred to are mainly ZAG, PKA, p-ERK, and CatSper. ZAG is involved in regulating the acrosome reaction of spermatozoa to increase their fertilization ability [77]. PKA mediates protein phosphorylation, which is important for sperm motility and male fertility because of its necessity in activating sperm capacitation [78,79]. At the same time, in mice, p-ERK can repair testicular damage caused by Biophenol exposure, as well as improve the sperm motility and sperm density, which is beneficial to semen quality [80]. Western blotting and

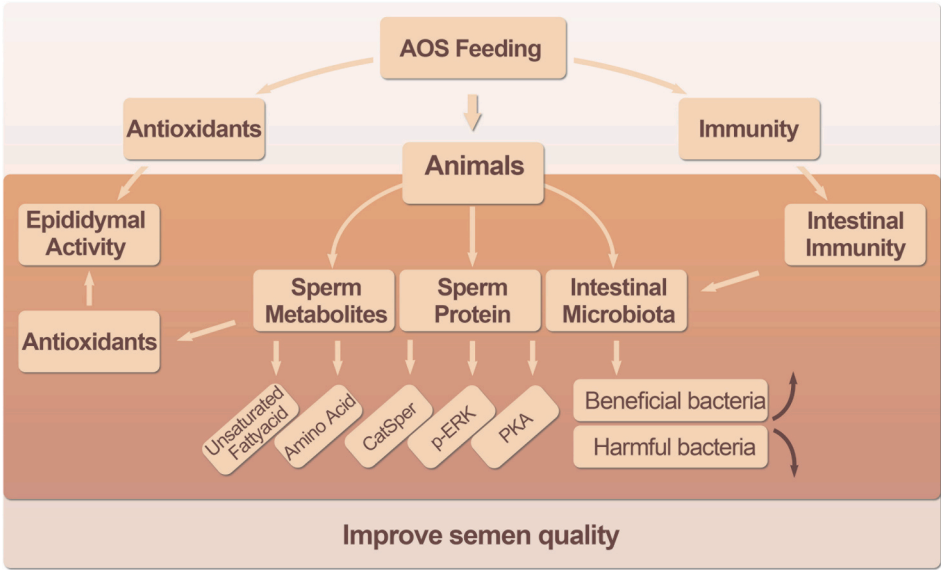

Fig. 4. The role of AOS on boar semen quality. AOS is a strong antioxidant which can reduce ROS in the epididymis. AOS can regulate the immune function of the intestine. Feeding AOS to the animals can improve sperm metabolites such as unsaturated fatty acids and sperm proteins, such as CatSper, p-ERK, and PKA, and it can change gut microbiota, as well as improve the overall semen quality.

immunocytochemistry showed that CatSper subunits are present in boar spermatozoa, primarily in the sperm neck, tail and cytoplasmic droplets [81]. The main mechanism involves the distribution of CatSper throughout the outer sperm membranes, preventing the binding with calcium, thereby improving boar sperm motility during capacitation [82]. Therefore, AOS regulates the semen quality of boars through improving the level of sperm proteins.

4.3. AOS changes gut microbes to increase semen quality

As the largest immunologically important organ in the digestive system, the intestine has emerged as a popular research hotspot [83]. The composition of intestinal microorganisms plays an important role in connecting host with diet. Therefore, the intestinal microbes and specific organs or axes between organs, such as the intestinal liver axis [84] and intestinal testicular axis [85], are more and more closely examined by many experts. Studies have shown that adding AOS to the basal diet of Duroc boars ($N = 9$, 24 month old with 300 kg body weight) can change the relative abundance of intestinal microbes, increasing beneficial bacteria and reducing harmful bacteria [72]. In the study by Zhou et al., at the phylum level, the relative abundance of *Bacteroidetes* increased and that of the *Firmicutes* decreased. At the genus level, *Butyricicoccus,* as a beneficial bacteria that can produce butadic acid which is benefit to spermatogenesis [86]. A Spearman associated analysis showed that *Bacteroidetes and Butyricicoccus* are positively correlated with sperm motility and sperm concentration. In contrast, *Firmicutes* are negatively correlated with sperm motility and sperm concentration [65]. At the same time, AOS can also reduce harmful bacteria such as *Prevotella*. Studies have shown that *Prevotella* appeared to exert a negative effect on sperm quality [87,88]. In addition, adding AOS to the diet of boars can increase the content of short-chain fatty acids (SCFAs) in the intestine, and specifically acetic acid and butyric acid are significantly improved [72]. SCFAs are small molecular compounds which can freely enter the testis through the blood and provide energy for the spermatogenesis [89]. Feeding AOS to mice changed the intestinal microbiota and sperm motility improved significantly [90]. Using fecal microbiota transplant (FMT) technology, by which bacteria were orally fed to the mice, the sperm motility and the relative abundance and colonization of beneficial bacteria such as *Bacteroidales* and *Bifidobacteriales* in the small intestine increased [75]. Therefore, AOS can increase the semen quality of boars by changing the microbial composition. On the one hand, this occurs through the increase of beneficial bacteria to promote the production of short-chain fatty acids, thereby promoting the interaction with the intestinal testicular axis. On the other hand, a reduction of intestinal harmful bacteria aims to inhibit the damage of spermatogenesis.

5. Conclusion and perspectives

Nowadays, large-scale boar studs have adopted high-intensive semen collection methods in order to improve reproduction efficiency. This lead to a decline in the semen quality and an increase in elimination rate. For a while now, the beneficial effects of AOS on boar semen quality have been subject to investigation. Important results of AOS have been achieved in the research of the intestinal testicular axis. A supplementation with AOS can indeed increase the semen quality through changing gut microbiota and metabolites in blood and sperm, as well as increasing sperm proteins that are related to spermatogenesis. Therefore, in this review we summarize the positive effects of AOS on intestinal microorganisms, blood metabolites, sperm metabolites and sperm proteins. However, the molecular mechanism by which AOS is regulating the semen quality of boars is not clear yet, and further research needs to be performed.

AOS is a natural additive with developmental value. Clarification of the relationship between structure and biological activity of AOS can help to prepare targeted AOS with specific functions, to be applied in the fields of food, medicine and feed additives. However, several bottlenecks still exist in the preparation and application of AOS. The structure-function relationships of oligosaccharides have not been well studied and are worthy of attention and exploration in the future. As heterogeneous oligosaccharides, AOS showed more diversities in structures than other homogeneous oligosaccharides including chito-oligosaccharides, manno-oligosaccharides and isomalto-oligosaccharides. The unique functions of AOS are strongly connected with the specific structures. The different structure can result in different functions of AOS. The current research on the relationship between the structure and biological activity of AOS is not in-depth enough. Most of the research focuses on the biological activity of AOS mixtures, and most of them are based on in vitro model studies, which limits the further development and utilization of AOS. In the future, we hope to clarify the detailed mechanism of AOS and how it can be used especially in specific agricultural settings, for example, in releasing heat stress or extended service lifespan of Duroc boars, in order to reduce the elimination rate in large scale boar stud. At the same time, it would be interesting to find targeted drugs containing AOS that can treat male infertility which is clinical manifestations of oligospermia. Now, some problems had been explored that AOS increased murine sperm concentration and motility, and rescued busulfan disrupted spermatogenesis, AOS can also improve the semen quality of diabetic mice model. Therefore, this review not only provides a theoretical basis for AOS as a feed additive in agriculture, but also provides ideas for solving human infertility problems.

CRediT authorship contribution statement

Yexun Zhou: Writing – original draft, Supervision. **Zeou Wei:** Writing – original draft, Supervision. **Yang Gao:** Writing – review & editing. **Hongfu Zhang:** Writing – review & editing, Project administration, Funding acquisition. **Martine Schroyen:** Writing – review & editing.

Declaration of competing interest

The remaining authors declare that the manuscript was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

No data was used for the research described in the article.

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References

[1] Y. Yu, et al., Biological activities and pharmaceutical applications of polysaccharide from natural resources: a review, Carbohydr. Polym. 183 (2018) 91–101.
[2] S. Basak, I. Mukherjee, T.K. Das, Injectable biocompatible RAFT mediated nitroxide nanogels: a robust ROS-reduction antioxidant approach, Colloids Surf. B Biointerfaces 236 (2024) 113790.
[3] T.K. Das, S. Ganguly, Revolutionizing food safety with quantum dot-polymer nanocomposites: from monitoring to sensing applications, Foods 12 (11) (2023).
[4] S. Lu, et al., Alginate oligosaccharides: the structure-function relationships and the directional preparation for application, Carbohydr. Polym. 284 (2022) 119225.
[5] H. Liao, H. Zhang, W. Chen, Differential physical, rheological, and biological properties of rapid in situ gelable hydrogels composed of oxidized alginate and gelatin derived from marine or porcine sources, J. Mater. Sci. Mater. Med. 20 (6) (2009) 1263–1271.
[6] S.N. Pawar, K.J. Edgar, Alginate derivatization: a review of chemistry, properties and applications, Biomaterials 33 (11) (2012) 3279–3305.

[7] M. Falkeborg, et al., Alginate oligosaccharides: enzymatic preparation and antioxidant property evaluation, Food Chem. 164 (2014) 185–194.
[8] X. Xu, et al., Immunomodulatory effects of alginate oligosaccharides on murine macrophage RAW264.7 cells and their structure-activity relationships, J. Agric. Food Chem. 62 (14) (2014) 3168–3176.
[9] Y. Zhang, et al., Alginate oligosaccharides ameliorate DSS-induced colitis through modulation of AMPK/NF-κB pathway and intestinal microbiota, Nutrients 14 (14) (2022).
[10] Y. Han, et al., Alginate oligosaccharide attenuates α2,6-sialylation modification to inhibit prostate cancer cell growth via the hippo/YAP pathway, Cell Death Dis. 10 (5) (2019) 374.
[11] G.F. Louis, et al., The effect of energy and protein intakes on boar libido, semen characteristics, and plasma hormone concentrations, J. Anim. Sci. 72 (8) (1994) 2051–2060.
[12] M. Schulze, et al., Influences on semen traits used for selection of young AI boars, Anim. Reprod. Sci. 148 (3–4) (2014) 164–170.
[13] W.E. Berndtson, Comparative reliability and sensitivity of different methods for assessing treatment effects on sperm production, Anim. Reprod. Sci. 105 (1–2) (2008) 5–22.
[14] M.L. Broekhuijse, et al., The value of microscopic semen motility assessment at collection for a commercial artificial insemination center, a retrospective study on factors explaining variation in pig fertility, Theriogenology 77 (7) (2012) 1466–1479.e3.
[15] A.L. Ruiz-Sánchez, et al., The predictive value of routine semen evaluation and IVF technology for determining relative boar fertility, Theriogenology 66 (4) (2006) 736–748.
[16] W.L. Flowers, Management of boars for efficient semen production, J. Reprod. Fertil. Suppl. 52 (1997) 67–78.
[17] C. Holt, et al., Objectively measured boar sperm motility parameters correlate with the outcomes of on-farm inseminations: results of two fertility trials, J. Androl. 18 (3) (1997) 312–323.
[18] R. Knox, et al., An update on north American boar stud practices, Theriogenology 70 (8) (2008) 1202–1208.
[19] M. Jung, K. Rüdiger, M. Schulze, In vitro measures for assessing boar semen fertility, Reprod. Domest. Anim. 50 (Suppl. 2) (2015) 20–24.
[20] A. Lopez Rodriguez, et al., Boar management and semen handling factors affect the quality of boar extended semen, Porcine Health Manag. 3 (2017) 15.
[21] X. Xu, et al., Morphological and proteomic analyses reveal that unsaturated guluronate oligosaccharide modulates multiple functional pathways in murine macrophage RAW264.7 cells, Mar. Drugs 13 (4) (2015) 1798–1818.
[22] J. Wan, et al., Alginate oligosaccharide alleviates enterotoxigenic Escherichia coli-induced intestinal mucosal disruption in weaned pigs, Food Funct. 9 (12) (2018) 6401–6413.
[23] M. Kurachi, et al., Comparison of the activities of various alginates to induce TNF-alpha secretion in RAW264.7 cells, J. Infect. Chemother. 11 (4) (2005) 199–203.
[24] M. Iwamoto, et al., Structure-activity relationship of alginate oligosaccharides in the induction of cytokine production from RAW264.7 cells, FEBS Lett. 579 (20) (2005) 4423–4429.
[25] M. Saigusa, et al., In vitro and in vivo anti-inflammatory activity of digested peptides derived from salmon myofibrillar protein conjugated with a small quantity of alginate oligosaccharide, Biosci. Biotechnol. Biochem. 79 (9) (2015) 1518–1527.
[26] J. Wan, et al., Alginate oligosaccharide enhances intestinal integrity of weaned pigs through altering intestinal inflammatory responses and antioxidant status, RSC Adv. 8 (24) (2018) 13482–13492.
[27] R. Zhou, et al., Alginate-derived oligosaccharide inhibits Neuroinflammation and promotes microglial phagocytosis of β-amyloid, Mar. Drugs 13 (9) (2015) 5828–5846.
[28] R. Zhou, et al., Anti-inflammatory activity of guluronate oligosaccharides obtained by oxidative degradation from alginate in lipopolysaccharide-activated murine macrophage RAW 264.7 cells, J. Agric. Food Chem. 63 (1) (2015) 160–168.
[29] T. Li, et al., Alginate oligosaccharides protect against fumonisin B1-induced intestinal damage via promoting gut microbiota homeostasis, Food Res. Int. 152 (2022) 110927.
[30] Y. Zhao, et al., Single-cell RNA sequencing analysis reveals alginate oligosaccharides preventing chemotherapy-induced mucositis, Mucosal Immunol. 13 (3) (2020) 437–448.
[31] M. Xing, et al., Advances in research on the bioactivity of alginate oligosaccharides, Mar. Drugs 18 (3) (2020).
[32] S. Aletaha, et al., M2000 (β-D-Mannuronic acid) as a novel antagonist for blocking the TLR2 and TLR4 downstream Signalling pathway, Scand. J. Immunol. 85 (2) (2017) 122–129.
[33] M. Hajivalili, et al., G2013 modulates TLR4 signaling pathway in IRAK-1 and TARF-6 dependent and miR-146a independent manner, Cell. Mol. Biol. (Noisy-le-Grand) 62 (4) (2016) 1–5.
[34] S.S. Mortazavi-Jahromi, et al., Effects of guluronic acid (G2013) on SHIP1, SOCS1 induction and related molecules in TLR4 signaling pathway, Int. Immunopharmacol. 55 (2018) 323–329.
[35] Y. Zhu, et al., Characterization of an extracellular biofunctional alginate lyase from marine Microbulbifer sp. ALW1 and antioxidant activity of enzymatic hydrolysates, Microbiol. Res. 182 (2016) 49–58.
[36] X. Fu, et al., Enzymatic preparation of low-molecular-weight Laminaria japonica polysaccharides and evaluation of its effect on modulating intestinal microbiota in high-fat-diet-fed mice, Front. Bioeng. Biotechnol. 9 (2021) 820892.
[37] E. Hernandez-Marin, A. Martínez, Carbohydrates and their free radical scavenging capability: a theoretical study, J. Phys. Chem. B 116 (32) (2012) 9668–9675.
[38] S.K. Tusi, et al., Alginate oligosaccharide protects against endoplasmic reticulum- and mitochondrial-mediated apoptotic cell death and oxidative stress, Biomaterials 32 (23) (2011) 5438–5458.
[39] J.J. Guo, et al., Alginate oligosaccharide prevents acute doxorubicin cardiotoxicity by suppressing oxidative stress and endoplasmic reticulum-mediated apoptosis, Mar. Drugs 14 (12) (2016).
[40] S. Li, N. He, L. Wang, Efficiently anti-obesity effects of unsaturated alginate oligosaccharides (UAOS) in high-fat diet (HFD)-fed mice, Mar. Drugs 17 (9) (2019).
[41] W. Zhu, et al., Effects of polymannuronate on performance, antioxidant capacity, immune status, cecal microflora, and volatile fatty acids in broiler chickens, Poult. Sci. 94 (3) (2015) 345–352.
[42] Y. Iwamoto, et al., Enzymatically depolymerized alginate oligomers that cause cytotoxic cytokine production in human mononuclear cells, Biosci. Biotechnol. Biochem. 67 (2) (2003) 258–263.
[43] S. Wee, W.R. Gombotz, Protein release from alginate matrices, Adv. Drug Deliv. Rev. 31 (3) (1998) 267–285.
[44] J. Chen, et al., Alginate oligosaccharide DP5 exhibits antitumor effects in osteosarcoma patients following surgery, Front. Pharmacol. 8 (2017) 623.
[45] K. Takahashi, et al., Local induction of a cytotoxic factor in a murine tumour by systemic administration of an antitumour polysaccharide, MGA, Br. J. Cancer 57 (2) (1988) 170–173.
[46] Z. Pan, et al., Sulfated alginate oligosaccharide exerts antitumor activity and autophagy induction by inactivating MEK1/ERK/mTOR signaling in a KSR1-dependent manner in osteosarcoma, Oncogenesis 11 (1) (2022) 16.
[47] S.Z. Han, et al., Semen quality and fertilization ability of myostatin-knockout boars, Theriogenology 135 (2019) 109–114.
[48] R. Sharma, et al., Effect of sperm storage and selection techniques on sperm parameters, Syst. Biol. Reprod. Med. 61 (1) (2015) 1–12.
[49] M. Quirino, et al., Sperm concentration of boar semen doses and sperm quality: novel perspectives based on the extender type and sperm resilience, Anim. Reprod. Sci. 255 (2023) 107293.
[50] R.K. Browne, et al., Sperm motility of externally fertilizing fish and amphibians, Theriogenology 83 (1) (2015) 1–13.
[51] G.K. Mahajan, R.T. Mahajan, A.Y. Mahajan, Improvement of sperm density in neem-oil induced infertile male albino rats by Ipomoea digitata Linn, J. Intercult. Ethnopharmacol. 4 (2) (2015) 125–128.
[52] D. Waberski, et al., Application of preserved boar semen for artificial insemination: past, present and future challenges, Theriogenology 137 (2019) 2–7.
[53] P. Chanapiwat, et al., Conception rate and litter size in multiparous sows after intrauterine insemination using frozen-thawed boar semen in a commercial swine herd in Thailand, J. Vet. Med. Sci. 76 (10) (2014) 1347–1351.
[54] A. Lesani, et al., Quantification of human sperm concentration using machine learning-based spectrophotometry, Comput. Biol. Med. 127 (2020) 104061.
[55] J.M. Morrell, Effect of colloid centrifugation on boar sperm quality during storage and function in in vitro fertilization, Theriogenology 137 (2019) 122–126.
[56] R. Bibi, et al., Protamines and DNA integrity as a biomarkers of sperm quality and assisted conception outcome, Andrologia 54 (6) (2022) e14418.
[57] J. Fedder, et al., Malformation rate and sex ratio in 412 children conceived with epididymal or testicular sperm, Hum. Reprod. 22 (4) (2007) 1080–1085.
[58] E. Kawakami, et al., Therapeutic effect of frequent injections of GnRH analogue in a beagle with knobbed acrosome abnormality of sperm, J. Vet. Med. Sci. 74 (2) (2012) 201–204.
[59] P. Buendía, et al., Morphometric characterization and classification of alpaca sperm heads using the sperm-class analyzer computer-assisted system, Theriogenology 57 (4) (2002) 1207–1218.
[60] R. Oko, F. Hrudka, Segmental aplasia of the mitochondrial sheath and sequelae induced by gossypol in rat spermatozoa, Biol. Reprod. 26 (1) (1982) 183–195.
[61] M.J. Callaghan, et al., Subacute ruminal acidosis reduces sperm quality in beef bulls, J. Anim. Sci. 94 (8) (2016) 3215–3228.
[62] L. du Plessis, J.T. Soley, Abaxial tail implantation in the emu, Dromaius novaehollandiae: morphological characteristics and origin of a rare avian sperm defect, Theriogenology 77 (6) (2012) 1137–1143.
[63] J. Li, et al., Exogenous spermidine effectively improves the quality of cryopreserved boar sperm, Anim. Sci. J. 94 (1) (2023) e13859.
[64] A. Salas-Huetos, et al., Diet and sperm quality: nutrients, foods and dietary patterns, Reprod. Biol. 19 (3) (2019) 219–224.
[65] H. Han, et al., Alginate oligosaccharides increase boar semen quality by affecting gut microbiota and metabolites in blood and sperm, Front. Microbiol. 13 (2022) 982152.
[66] S.A. Keys, W.F. Zimmerman, Antioxidant activity of retinol, glutathione, and taurine in bovine photoreceptor cell membranes, Exp. Eye Res. 68 (6) (1999) 693–702.
[67] R.C. Jones, To store or mature spermatozoa? The primary role of the epididymis, Int. J. Androl. 22 (2) (1999) 57–67.
[68] R. Chianese, R. Pierantoni, Mitochondrial reactive oxygen species (ROS) production alters sperm quality, Antioxidants (Basel) 10 (1) (2021).
[69] M. Taşkıran, Is there an association between dietary antioxidant levels and sperm parameters in male infertility? Cureus 15 (8) (2023) e44339.
[70] B. Hosseini, et al., The effect of Omega-3 fatty acids, EPA, and/or DHA on male infertility: a systematic review and meta-analysis, J. Diet. Suppl. 16 (2) (2019) 245–256.
[71] G. Chen, et al., Lysine acetylation participates in boar spermatozoa motility and acrosome status regulation under different glucose conditions, Theriogenology 159 (2021) 140–146.

[72] Y. Zhou, et al., Alginate oligosaccharide extends the service lifespan by improving the sperm metabolome and gut microbiota in an aging Duroc boars model, Front. Cell. Infect. Microbiol. 13 (2023) 1308484.
[73] J. Wang, et al., Exposure to dibutyl phthalate and reproductive-related outcomes in animal models: evidence from rodents study, Front. Physiol. 12 (2021) 684532.
[74] J.S. Johnson, et al., In utero heat stress alters postnatal phenotypes in swine, Theriogenology 154 (2020) 110–119.
[75] P. Zhang, et al., Improvement in sperm quality and spermatogenesis following faecal microbiota transplantation from alginate oligosaccharide dosed mice, Gut 70 (1) (2021) 222–225.
[76] Y. Zhao, et al., Alginate oligosaccharides improve germ cell development and testicular microenvironment to rescue busulfan disrupted spermatogenesis, Theranostics 10 (7) (2020) 3308–3324.
[77] Y. Liu, et al., Con A-binding protein Zn-α2-glycoprotein on human sperm membrane is related to acrosome reaction and sperm fertility, Int. J. Androl. 35 (2) (2012) 145–157.
[78] Z. Liang, et al., AKAP3 mediated type I PKA signaling is required for mouse sperm Hyperactivation and fertility, Biol. Reprod. (2023).
[79] C. Baro Graf, et al., Everything you ever wanted to know about PKA regulation and its involvement in mammalian sperm capacitation, Mol. Cell. Endocrinol. 518 (2020) 110992.
[80] J. Li, et al., Exposure to bisphenol a (BPA) in Wistar rats reduces sperm quality with disruption of ERK signal pathway, Toxicol. Mech. Methods 26 (3) (2016) 180–188.
[81] L. Tamburrino, et al., The CatSper calcium channel in human sperm: relation with motility and involvement in progesterone-induced acrosome reaction, Hum. Reprod. 29 (3) (2014) 418–428.
[82] A. Vicente-Carrillo, M. Álvarez-Rodríguez, H. Rodríguez-Martínez, The CatSper channel modulates boar sperm motility during capacitation, Reprod. Biol. 17 (1) (2017) 69–78.
[83] A.M. Mowat, W.W. Agace, Regional specialization within the intestinal immune system, Nat. Rev. Immunol. 14 (10) (2014) 667–685.
[84] D. Porras, et al., Protective effect of quercetin on high-fat diet-induced non-alcoholic fatty liver disease in mice is mediated by modulating intestinal microbiota imbalance and related gut-liver axis activation, Free Radic. Biol. Med. 102 (2017) 188–202.
[85] Z. Wang, et al., Salidroside ameliorates furan-induced testicular inflammation in relation to the gut-testis Axis and intestinal apoptosis, J. Agric. Food Chem. 71 (46) (2023) 17968–17987.
[86] Y. Lin, et al., The improvement of semen quality by dietary Fiber intake is positively related with gut microbiota and SCFA in a boar model, Front. Microbiol. 13 (2022) 863315.
[87] Y. Zhou, et al., Taxifolin increased semen quality of Duroc boars by improving gut microbes and blood metabolites, Front. Microbiol. 13 (2022) 1020628.
[88] L. Farahani, et al., The semen microbiome and its impact on sperm function and male fertility: a systematic review and meta-analysis, Andrology 9 (1) (2021) 115–144.
[89] J. Wu, et al., Mixed probiotics modulated gut microbiota to improve spermatogenesis in bisphenol A-exposed male mice, Ecotoxicol. Environ. Saf. 270 (2024) 115922.
[90] C. Zhang, et al., Rescue of male fertility following faecal microbiota transplantation from alginate oligosaccharide-dosed mice, Gut 70 (11) (2021) 2213–2215.