Maternal chitosan oligosaccharide supplementation during late gestation and lactation affects offspring growth

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ABSTRACT
Chitosan oligosaccharide (COS), a natural compound derived from chitin, has growth promotion property. However, the nature of the relationship between maternal COS supplementation and offspring growth is not yet understood. Therefore, we intended to determine the effects of maternal COS supplementation in late gestation and lactation on the offspring growth performance. Twenty-four pregnant Yorkshire sows were distributed into two equal groups (n = 12) and fed either with a basal diet (control group) or a basal diet containing 100 mg/kg COS, from gestation day 85 until lactation day 21. Serum samples were collected from six sows of average body weight, from each group on farrowing day (lactation day 1) and lactation day 21, whereascolostrum and milk samples were respectively obtained on lactation days 1 and 21. We found that maternal COS supplementation significantly increased (p < .05) the average piglet weaning weight per litter. Supplementation of COS in the sow diet elevated (p < .05) the serum interleukin-10, immunoglobulin A (IgA) and IgM concentrations, as well as total antioxidant capacity. Moreover, higher (p < .05) colostrum IgM and milk lactose contents were simultaneously observed in COS-treated sows compared to the control sows. In summary, COS supplementation during late gestation and lactation can boost antioxidant capacity and humoural immunity of sows, and contributes to improving colostrum and milk quality, ultimately, enhancing the offspring growth performance.

Introduction
Antibiotics, as growth promoters and therapeutic drugs, have been used in animal production for many years (Gong et al. 2014). Nevertheless, the misuse of antibiotics has caused many problems, including the emergence of bacteria resistant to antibiotics and the heightened concern of drug residues in animal products (Monroe and Polk 2000; Smith et al. 2002). For these reasons, considerable attention has been focussed on the development of alternatives to antibiotics, to maintain livestock performance (Wan, Li, Chen, Yu, Cheng, et al. 2016; Wan, Li, Chen, Yu, Zheng, et al. 2016).

Chitosan is a natural-based biopolymer derived from chitin, which is the second most abundant polymer in nature (Hajji et al. 2014; Wan, Jiang, Xu, et al. 2017). However, its insolubility and high viscosity limit its application as a nutrient source. Conversely, chitosan oligosaccharide (COS), the soluble degradation product of chitosan, has a low molecular weight, good solubility and low viscosity and, furthermore, may be absorbed more readily by animals and humans than chitosan (Muanprasat and Chatsudthipong 2017). Studies have shown that COS possesses multiple functional properties, including anti-oxidation (Fernandes et al. 2010), anti-inflammation (Lee et al. 2009) and immune-enhancing characteristics (Okamoto et al. 2003). Researches have also revealed that COS can be used as a substitute to antibiotics and confer marked health benefits in animals (Yang et al. 2012; Xie et al. 2015; Suthongsa et al. 2017).

Maternal nutritional and health status during the transition from gestation to lactation are important in maintaining suckling piglet health and growth (Le Dividich 2006; Hansen et al. 2012). Thus, reasonable nutritional supplementation for sows, especially in lactation, is crucial for enhancing offspring growth. Currently, dietary supplementation of COS provides a novel strategy to improve the reproductive performance of sows (Cheng et al. 2015; Wan, Yang, et al. 2016).
However, further investigations are needed to clarify the connection between maternal COS supplementation during late gestation and lactation with offspring growth.

Herein, we postulated that supplementing COS in the sow diet during late gestation and lactation might improve the sow health status and thereby stimulate suckling piglet growth, by influencing the colostrum and milk nutrient compositions. To test this hypothesis, we investigated the changes in serum antioxidant and immune parameters, and the variations of colostrum and milk compositions in sows supplemented with COS, as well as alterations in suckling piglet growth performance. Our results will aid in developing cost-effective strategies to enhance litter performance in swine and also have important implications for improving newborn growth in other mammals.

Materials and methods
All experimental procedures used in this study were approved by the Animal Management Rules of the Ministry of Health of the People’s Republic of China and the Animal Care and Use Committee of Sichuan Agricultural University (Chengdu, China).

Animals and treatments
Twenty-four pregnant Yorkshire sows with three parities were selected from a commercial pig farm (Leshan, China) and allotted to two dietary treatments (n = 12). The dietary treatments were as follows: (1) a corn-soybean basal with no COS (control, CON) and (2) the basal diet supplemented with 100 mg/kg COS (termed COS). All sows were individually housed in gestation crates (1.5 × 2.0 m) and transferred to individual farrowing crates (2.0 × 2.5 m) on day 108 of gestation.

Table 1 shows the compositions of the gestation and lactation diets, which were formulated to meet all of the requirements for gestating and lactating sows, recommended by the National Research Council (2012). COS, with an average molecular weight ≤1000 Da, was obtained from the Dalian Institute of Chemical Physics, Chinese Academy of Sciences (Dalian, China). Before delivery, sows were fed 2.8 kg gestation diets per day. On farrowing day (lactation day 1), sows initially received 1 kg of their lactation diets and the ration, which was then increased by 0.8 kg/d on days 1 and 2, and by 1 kg/day on days 3 and 4, for each sow (Quiniou and Noblet 1999). All sows were fed their respective diets twice daily at 08.00 and 18.00 h from gestation day 85 to lactation day 21, and water was provided ad libitum to all sows and piglets via individual nipple drinkers, throughout the course of the experiment. Additionally, within each farrowing crate, the piglets’ area was heated with infra-red lamps to maintain the ambient temperature at approximately 30 °C.

Litter performance measurement
Piglets were cross-fostered within treatments shortly after birth to standardise litter size to 10 and had no access to creep feed until weaning (lactation day 21). Piglets were individually weighed after cross-fostering on days 1 and 21 of lactation, and average daily body weight gain per litter was calculated.

Sample collection
On lactation days 1 (1 h after farrowing) and 21, six sows per group, of average body weight, were selected and blood samples (10 mL) were collected by anterior vena cava puncture. Serum samples were then obtained by centrifuging the blood samples at 3000 × g/4 °C for 10 min and were subsequently stored at −20 °C until analysis. On lactation days 1 (between 1–3 h after farrowing) and 21, after blood collection, colostrum and milk samples (50 mL) were manually collected from all functional mammary glands of the same six selected sows per group. Immediately,
all colostrum and milk samples from the same sow were mixed and stored at −20 °C, for later analyses.

**Serum antioxidant status evaluation**

Serum antioxidant status, as malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and total antioxidant capacity (T-AOC), was evaluated using a multi-mode microplate reader (Spectra Max M2, Molecular Devices, Sunnyvale, CA, USA). All antioxidant-related kits were provided by the Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

**MDA content analysis**

MDA content was assayed by reaction of the serum samples with thiobarbituric acid in an acidic medium at 95°C for 30 min, to generate a pink product that was spectrophotometrically determined at 532 nm (Livingstone et al. 1990). The results were expressed in nmol per millilitre of serum.

**SOD activity analysis**

SOD activity was measured spectrophotometrically at 550 nm, as described elsewhere (Wan, Jiang, et al. 2016). This technique involves the decrease of the product (superoxide ions) of the xanthine/xanthine oxidase system and the formation of red formazan by reacting with 2-(4-iodophenyl)3-(4-nitrophenol)-5-phenyltetrazolium chloride. SOD activity was presented as units per millilitre of serum, where 1 U of SOD denotes 50% inhibition of superoxide ion production in the reaction.

**CAT activity analysis**

CAT activity was examined using the colorimetric method provided by Aebi (1984). The enzymatic reaction was terminated by the addition of ammonium molybdate, which generated a light-yellow composite that could be measured at 405 nm. CAT activity was expressed as units per mL of serum. One unit of CAT was defined as the amount of enzyme needed to decompose 1 mmol/L H₂O₂/s at 37 °C.

**GSH-Px activity analysis**

GSH-Px activity was determined by quantifying the rate of H₂O₂-induced oxidation of GSH to oxidised glutathione (GSSG) (Zhang et al. 2008). A yellow product with absorbance at 412 nm is formed as GSH reacts with 5,5'-dithiobis-(2-nitrobenzoic acid). Serum GSH-Px activity is expressed as units per millilitre serum, where 1 U of GSH-Px is defined as the amount of enzyme that decreases 1 mmol/L GSH/min.

**T-AOC analysis**

The determination of T-AOC would enable the evaluation of the total activities of several parameters (Miller et al. 1993), including the polyphenol complexes, protein thiol groups, vitamin C, vitamin E and glutathione, all of which can convert Fe³⁺ to Fe²⁺. Fe²⁺ can then combine with phenanthroline to form stable and coloured chelates. T-AOC was estimated at 520 nm and expressed as units per millilitre serum. Here, 1 U represents a 0.01 increase in the absorbance value in 1 min.

**Serum cytokines and immunoglobulins assay**

Serum immunoglobulins (immunoglobulin A (IgA), IgG and IgM) and cytokines (interleukin-1 (IL-1), IL-6 and tumour necrosis factor-α (TNF-α)) concentrations were measured using commercially available ELISA kits from R&D Systems (Minneapolis, MN, USA), according to the manufacturer’s instructions. The cytokine and immunoglobulin concentrations were presented as picogram per millilitre (pg/mL) and microgram per millilitre (μg/mL) of serum, respectively.

**Colostrum and milk analyses**

Before the analysis, aliquots of colostrum and milk were thawed at room temperature for 15 min. Colostrum and milk compositions, including fat, protein, lactose and solids-not-fat, were analysed with a fully automatic milk analyser (MilkoScan FT1, FOSS Electric, Hillerød, Denmark), as detailed previously (Liu et al. 2014). Whey samples were obtained from colostrum and milk, after centrifugation at 3000 × g/4 °C, for 30 min, to remove free fat. Immunoglobulins in the whey were analysed in line with the measurements of serum immunoglobulins described above.

**Statistical analysis**

All data were subjected to a Student’s t-test, using SAS 9.0 (SAS Inst. Inc., Cary, NC, USA). Data are presented as means ± standard errors. An individual sow or its litter was used as an experimental unit. Statistical significance was set at p < .05, whereas .05 < p < .10 was considered a trend towards significance.
**Results**

**Litter performance**

Table 2 presents the sow litter growth performance between the two groups. Average piglet weaning weight per litter was significantly increased ($p < .05$) through COS intervention in the sow diet.

### Serum antioxidant-related indicators

The antioxidant-related indicators in the serum after COS supplementation are listed in Table 3. The inclusion of COS in the sow diet promoted a 43.79% increase ($p < .05$) in the serum CAT activity and 29.88% decrease ($p < .05$) in serum MDA content at lactation day 1, compared to the control group. Serum T-AOC was enhanced ($p < .05$) by 48.23 and 14.20%, respectively, after COS administration at days 1 and 21 of lactation. Compared to the control sows, COS supplementation tended to increase ($0.05 < p < .10$) serum GSH-Px activity, only at lactation day 1. At all of the selected lactation time points, no effect ($p > .10$) on serum SOD activity was noted relative to the control group.

### Serum immune parameters

At lactation day 1, COS supplementation had a significant impact ($p < .05$) on IL-10, IgA and IgM concentrations, which were higher in the COS group than CON group, as seen in Table 4. In comparison to the CON sows, the IL-6 and IgG levels on lactation day 1, as well as IgM level on lactation day 21, had a tendency ($0.05 < p < .10$) to increase in COS-supplemented sows. In addition, no significant differences ($p > .10$) were observed for serum IL-1 and TNF-α levels throughout the experimental period.
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fat content, while it did not affect (.05
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the colostrum did not differ (.05

**Table 5.** Effects of chitosan oligosaccharide supplementation
during late gestation and lactation on the colostrum and milk compositions of sows**.

| Items               | CON       | COS       | p Value |
|---------------------|-----------|-----------|---------|
| Colostrum           |           |           |         |
| Protein, g/kg       | 53.90 ± 6.69 | 61.70 ± 1.45 | .078    |
| Fat, g/kg           | 62.77 ± 6.42 | 63.72 ± 3.84 | .901    |
| Lactose, g/kg       | 80.43 ± 4.53 | 82.43 ± 3.06 | .722    |
| Solids-not-fat, g/kg| 128.07 ± 6.85 | 153.33 ± 5.72 | .018    |
| Milk                |           |           |         |
| Protein, g/kg       | 36.17 ± 2.20 | 37.08 ± 1.32 | .728    |
| Fat, g/kg           | 61.48 ± 3.52 | 88.40 ± 11.12 | .060    |
| Lactose, g/kg       | 44.12 ± 2.82 | 56.10 ± 3.57 | .025    |
| Solids-not-fat, g/kg| 85.44 ± 5.38 | 101.82 ± 2.17 | .022    |

* p < .05 versus the CON group.

aValues are means of six replicates per treatment.

bCON: a corn-soybean basal diet; COS: chitosan oligosaccharide (the basal diet supplemented with 100 mg/kg chitosan oligosaccharide).

**Table 6.** Effects of chitosan oligosaccharide supplementation
during late gestation and lactation on the colostrum and milk immunoglobulin levels of sows**.

| Items       | CON       | COS       | p Value |
|-------------|-----------|-----------|---------|
| Colostrum   |           |           |         |
| IgAc, g/L   | 5.66 ± 0.38 | 5.79 ± 0.24 | .777    |
| IgGd, g/L   | 24.26 ± 0.97 | 20.74 ± 2.22 | .054    |
| IgMg, g/L   | 3.27 ± 0.22 | 4.76 ± 0.30 | .004    |
| Milk        |           |           |         |
| IgAc, g/L   | 1.40 ± 0.11 | 1.51 ± 0.13 | .560    |
| IgGd, g/L   | 7.29 ± 0.64 | 9.41 ± 0.73 | .061    |
| IgMg, g/L   | 1.71 ± 0.09 | 1.75 ± 0.09 | .769    |

** p < .01 versus the CON group.

Values are means of six replicates per treatment.

bCON: a corn-soybean basal diet; COS: chitosan oligosaccharide (the basal diet supplemented with 100 mg/kg chitosan oligosaccharide).

cIgA: immunoglobulin A.
dIgG: immunoglobulin G.
eIgM: immunoglobulin M.

**Colostrum and milk compositions**

Table 5 shows that sows fed with COS had a higher (p < .05) solids-not-fat content both in colostrum and milk. After COS supplementation, lactose content in the colostrum did not differ (p > .10) between the two treatments, but it appeared to be higher (p < .05) in the milk of the COS compared to CON sows. In the milk, COS ingestion tended to raise (.05 < p < .10) the fat content, while it did not affect (p > .10) this parameter in the colostrum. Moreover, COS supplementation tended to improve (.05 < p < .10) the colostrum protein content, without affecting (p > .10) the milk protein content.

**Colostrum and milk immunoglobulin levels**

The data in Table 6 reveal dietary COS supplementation enhanced (p < .05) the IgG level in colostrum, but not (p > .10) in milk. Sows fed COS tended to have increased (.05 < p < .10) IgG levels in the colostrum and milk. Furthermore, dietary treatments had no effects (p > .10) on the colostrum and milk IgA levels.

**Discussion**

It has already been established that pregnant sows have an elevated oxidative stress during late gestation and this can be sustained until the lactation ends (Berchieri-Ronchi et al. 2011). The increased oxidative stress is responsible for impaired milk production of sows, which directly affects the health and growth of nursing piglets (Kim et al. 2013). There is evidence that such oxidative stress problems can be alleviated by supplementation with certain antioxidants (Xie et al. 2016). Therefore, we surveyed a few serum antioxidant-related parameters, including SOD, CAT, T-AOC, GSH-Px and MDA, to confirm whether COS can improve the antioxidant status of sows. Interestingly, we observed a decreased serum MDA content, which indicates that lipid peroxidation could be reduced by COS (Feng et al. 2014). CAT is implicated as an essential defence enzyme against the potential toxicity of hydroxyl radicals, and T-AOC reflects the total antioxidant capacity of the organism (Fang et al. 2016; Wan, Zhang, et al. 2017). As anticipated, we found that COS ingestion increased serum CAT activity and T-AOC. These associated results jointly indicated that COS supplementation could enhance the antioxidant defence capacity of sows against oxidative stress (Wan, Zhang, et al. 2017).

Cytokines play a crucial role in the immune and inflammatory responses and their balance is important for protection against infection (Praveena et al. 2010). At present, little is known about the effects of COS supplementation from late gestation to lactation on serum cytokine contents in sows. Hence, we examined whether COS directly influences the serum cytokines of sows. As shown, COS elevated the serum IL-10 concentration in sows. Anti-inflammatory cytokines, like IL-10, prevent overactivation of the immune response and suppress the production of pro-inflammatory cytokines, to maintain immune homeostasis (Opal and Depalo 2000). Thus, COS ingestion could mitigate the inflammatory response in sows, through increasing the serum IL-10 concentration. Furthermore, higher serum IgA and IgM levels were also observed in sows from the COS group compared to those in the control group, suggesting that COS could be conducive to mounting an immune response to infection in sows (Wan, Yang, et al. 2016). According to the obtained results, we deduced that addition of COS to the sow diet during late gestation and lactation could help in
maintaining an excellent immune status in sows, through modulating serum cytokine and immunoglobulin levels.

Piglets are born agammaglobulinemic (i.e. without immunoglobulins) because circulating immunoglobulins in sows cannot cross the placental barrier (Le Dividich et al. 2005; Salmon et al. 2009). Colostrum is a unique mammary secretion not only regarding the supply of energy and specific nutrients to the neonate but also bioactive molecules, such as immunoglobulins (Theil et al. 2014). Consequently, intake of colostrum is crucial for the neonatal piglet to obtain passive immunity (Rooke and Bland 2002). In this study, we detected that the IgM level in the colostrum was higher in the COS group than CON group. It was, therefore, reasonable to assume that piglet passive immunity at the postnatal period could be improved by supplementing COS to lactating sows. Another alluring finding is that COS supplementation increased the lactose content in milk. Thus, the improvements in milk lactose secretion may confer an advantage to the nursing piglet because of the greater amount of energy provided by the milk (Martin et al. 2016). Overall, supplementation of the sow diet with COS during late gestation and lactation could increase the output of breast milk nutrients, thereby contributing, at least partially, to a greater litter growth performance during lactation.

Conclusions

We evidenced that the offspring growth was accelerated by adding COS to the sow diet during late gestation and lactation. A possible reason is that supplementation with COS during late gestation and lactation beneficially modified the antioxidant and immune statuses of sows, resulting in the improved colostrum and milk compositions. These findings offer new insights into how a COS-supplemented diet during late gestation and lactation plays an important role in improving offspring growth performance.

Acknowledgements

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Disclosure statement

The authors declare that they have no competing interests.

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References

Aebi H. 1984. Catalase in vitro. Meth Enzymol. 105:121–126. Berchieri-Ronchi CB, Kim SW, Zhao Y, Correa CR, Yeum KJ, Ferreira ALA. 2011. Oxidative stress status of highly prolific sows during gestation and lactation. Animal. 5:1774–1779.

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References

Aebi H. 1984. Catalase in vitro. Meth Enzymol. 105:121–126. Berchieri-Ronchi CB, Kim SW, Zhao Y, Correa CR, Yeum KJ, Ferreira ALA. 2011. Oxidative stress status of highly prolific sows during gestation and lactation. Animal. 5:1774–1779. Cheng LK, Wang LX, Xu QS, Huang LJ, Zhou DS, Li Z, Li SG, Du YG, Yin H. 2015. Chitooligosaccharide supplementation improves the reproductive performance and milk composition of sows. Livest Sci. 174:74–81.

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Lee SH, Seneviratne M, Ahn CB, Kim SK, Je JY. 2009. Factors affecting anti-inflammatory effect of chitooligosaccharides in lipopolysaccharides-induced RAW264.7 macrophage cells. Bioorg Med Chem Lett. 19:6655–6658.

Liu ST, Hou WX, Cheng SY, Shi BM, Shan AS. 2014. Effects of dietary citric acid on performance, digestibility of calcium and phosphorus, milk composition and immunoglobulin in sows during late gestation and lactation. Anim Feed Sci Technol. 191:67–75.

Livingstone DR, Martinez PG, Michel X, Narbonne J, O’hara S, Ribera D, Winston G. 1990. Oxyradical production as a pollution-mediated mechanism of toxicity in the common mussel, Mytilus edulis L., and other molluscs. Funct Ecol. 4:415–424.

Martin CR, Ling PR, Blackburn GL. 2016. Review of infant feeding: key features of breast milk and infant formula. Nutrients. 8:279.

Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. 1993. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin Sci. 84:407–412.

Monroe S, Polk R. 2000. Antimicrobial use and bacterial resistance. Curr Opin Microbiol. 3:496–501.

Muanprasat C, Chatsudthipong V. 2017. Chitosan oligosaccharide biological activities and potential therapeutic applications. Pharmacol Therapeut. 170:80–97.

National Research Council. 2012. Nutrient requirements of swine. 11th ed. Washington (DC): National Academies Press.

Okamoto Y, Inoue A, Miyatake K, Ogihara K, Shigemasa Y, Minami S. 2003. Effects of chitin/chitosan and their oligomers/monomers on migrations of macrophages. Macromol Biosci. 3:587–590.

Opal SM, Depalo VA. 2000. Anti-inflammatory cytokines. Chest. 117:1162–1172.

Praveena PE, Periasamy S, Kumar AA, Singh N. 2010. Cytokine profiles, apoptosis and pathology of experimental Pasteurella multocida serotype A1 infection in mice. Res Vet Sci. 89:332–339.

Quiniou N, Noblet J. 1999. Influence of high ambient temperatures on performance of multiparous lactating sows. J Anim Sci. 77:2124–2134.

Rooke JA, Bland IM. 2002. The acquisition of passive immunity in the new-born piglet. Livest Prod Sci. 78:13–23.

Salmon H, Berri M, Gerdts V, Meurens F. 2009. Humoral and cellular factors of maternal immunity in swine. Dev Comp Immunol. 33:384–393.

Smith DL, Harris AD, Johnson JA, Silbergeld EK, Morris JG. 2002. Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. Proc Natl Acad Sci USA. 99:6434–6439.

Suthongsa S, Pichyangkura R, Kalandakanond-Thongsong S, Thongsong B. 2017. Effects of dietary levels of chito-oligosaccharide on ileal digestibility of nutrients, small intestinal morphology and crypt cell proliferation in weaned pigs. Livest Sci. 198:37–44.

Theil PK, Lauridsen C, Quesnel H. 2014. Neonatal piglet survival: impact of sow nutrition around parturition on fetal glycogen deposition and production of colostrum and transient milk. Animal. 8:1021–1030.

Wan J, Jiang F, Xu QS, Chen DW, He J. 2016. Alginic acid oligosaccharide accelerates weaned pig growth through regulating antioxidant capacity, immunity and intestinal development. RSC Adv. 6:87026–87035.

Wan J, Jiang F, Xu QS, Chen DW, Yu B, Huang ZQ, Mao XB, Yu J, He J. 2017. New insights into the role of chitosan oligosaccharide in enhancing growth performance, antioxidant capacity, immunity and intestinal development of weaned pigs. RSC Adv. 7:9669–9679.

Wan J, Jiang F, Zhang J, Xu QS, Chen DW, Yu B, Mao XB, Yu J, Luo YH, He J. 2017. Amniotic fluid metabolomics and biochemistry analysis provides novel insights into the diet-regulated foetal growth in a pig model. Sci Rep. 7:44782.

Wan J, Li Y, Chen DW, Yu B, Chen G, Zheng P, Mao XB, Yu J, He J. 2016. Recombinant plectasin elicits similar improvements in the performance and intestinal mucosa growth and activity in weaned pigs as an antibiotic. Anim Feed Sci Technol. 211:216–226.

Wan J, Li Y, Chen DW, Yu B, Zheng P, Mao XB, Yu J, He J. 2016. Expression of a tandemly arrayed plectasin gene from Pseudoplectania nigrella in Pichia pastoris and its antimicrobial activity. J Microbiol Biotechnol. 26:461–468.

Wan J, Yang KY, Xu QS, Chen DW, Yu B, Luo YH, He J. 2016. Dietary chitosan oligosaccharide supplementation improves foetal survival and reproductive performance in multiparous sows. RSC Adv. 6:70715–70722.

Wan J, Zhang J, Chen DW, Yu B, He J. 2017. Effects of alginate oligosaccharide on the growth performance, antioxidant capacity and intestinal digestion-absorption function in weaned pigs. Anim Feed Sci Technol. 234:118–127.

Xie CY, Guo XY, Long CM, Fan ZY, Xiao DF, Ruan Z, Deng ZY, Wu X, Yin YL. 2015. Supplementation of the sow diet with chitosan oligosaccharide during late gestation and lactation affects hepatic gluconeogenesis of suckling piglets. Anim Reprod Sci. 159:109–117.

Xie CY, Wu X, Long CM, Wang QH, Fan ZY, Li SM, Yin YL. 2016. Chitosan oligosaccharide affects antioxidant defense capacity and placental amino acids transport of sows. BMC Vet Res. 12:243.

Yang CM, Ferket PR, Hong QH, Zhou J, Cao GT, Zhou L, Chen AG. 2012. Effect of chito-oligosaccharide on growth performance, intestinal barrier function, intestinal morphology and cecal microflora in weaned pigs. J Anim Sci. 90:2671–2676.

Zhang XD, Zhu YF, Cai LS, Wu TX. 2008. Effects of fasting on the meat quality and antioxidant defenses of market-size farmed large yellow croaker (Pseudosciaena crocea). Aquaculture. 280:136–139.