

*Review Article*

## **SUPPLEMENTATION OF SHRIMP SHELL MEAL CONTAINING CHITOSAN AND ITS OLIGOSACCHARIDE DERIVATIVES (CHITO-OLIGOSACCHARIDE) AS A SOURCE OF PREBIOTIC IN CROSSBRED PIGS**

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**Abstract:** This paper summarizes the available information on the effects of dietary chitosan and its oligosaccharide derivatives on performance and metabolic response in pigs, i.e. hematological, biochemical, and immunological blood characteristics, microbiological profile of intestines, intestinal morphology, and digestibility of nutrients, as well as on the quality of meat. The results of most of the experiments presented in this review indicate that chitosan used as a feed additive for pigs has some beneficial, biological effects, including immunomodulatory, antioxidative, antimicrobial, and hypocholesterolaemic properties. These properties of chitosan, unlike many other kinds of feed additives, were often reflected in improved growth performance (body weight gain and/or feed conversion ratio) of pigs. Chitosan, obtained from chito-oligosaccharides is widely distributed in exoskeleton of shrimp, which is nontoxic biodegradable carbohydrate. Since chitosan contains reactive functional groups, i.e., amino acids and hydroxyl groups, it is characterised by antimicrobial, anti-inflammatory, antioxidative, antitumor, immunostimulatory, and hypocholesterolaemic properties when fed as dietary additive for farm animals.

**Keywords:** Chitosan, pigs, Shrimp shell meal.

### **INTRODUCTION**

Demand for animal protein for human consumption is rising globally at an unprecedented pace. Modern pig production practices that are associated with regular use of antibiotics as growth promoters or to control diseases (Yang *et al.*, 2015) which contribute to the spread of drug-resistant pathogens in both livestock and humans, posing a significant public health threat (Van der Fels-Klerx *et al.*, 2011). However, these negative effects of antibiotics have become increasingly prominent and consumers are concerned about antibiotic residues in meat products (Vondruskova *et al.*, 2010). Moreover with the ban of antibiotic growth promoters by several countries (Simon *et al.*, 2003), extensive research work has become a continuous process leading to the identification of many potential alternative growth promoters such as acidifiers, probiotics, prebiotics etc to reduce or eliminate the use of antibiotics in animal feeds.

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In recent years, multiple reports have described the beneficial effects of dietary oligosaccharides such as chito-oligosaccharides by enhancing host health status, modulating the intestinal microflora (Yang *et al.*, 2012; Xu *et al.*, 2014; Yugandharkumar *et al.*, 2017) and improving the small intestine morphological structure (Swiatkiewicz *et al.*, 2015). Furthermore, dietary chito-oligosaccharides, serve not only as growth promoters for helpful bacteria but also effectively inhibit the growth and activity of pathogenic microorganisms (Xuet *et al.*, 2012).

Chitosan, obtained from chito-oligosaccharides is (Lodhi *et al.*, 2014) the second most abundant carbohydrate polymer in nature (Ameh *et al.*, 2015; Azuma *et al.*, 2015; Krishnaveni and Ragunathan, 2015) after cellulose and is widely distributed in exoskeleton of shrimp and crab (Arafat *et al.*, 2015; Azuma *et al.*, 2015) which is nontoxic, biodegradable carbohydrate polymer (Goiri *et al.*, 2010), contains amino and hydroxyl groups (Ramya *et al.*, 2012; Arafat *et al.*, 2015), which gives chitosan many biological activities such as antimicrobial activity (Benhabiles *et al.*, 2012; Azuma *et al.*, 2015), fungistatic and hemostatic activity (Hui *et al.*, 2012), hypocholesterolemic activity (Kerch, 2015), antioxidant activity (Trung and Bao, 2015), antitumor (Yogeshkumar *et al.*, 2013; Azuma *et al.*, 2015), immune-stimulatory effect (Wu *et al.*, 2015) and binds to mammalian and microbial cells aggressively (Dutta *et al.*, 2004) when fed as dietary additive for farm animals. These properties of chitosan, unlike many other kinds of feed additives, were often reflected in improved growth performance of animals (Xu *et al.*, 2014; Swiatkiewicz *et al.*, 2015). Apart from this using shrimp waste meal containing chitosan as feedstuff is a part of waste management in the shrimp processing industry.

The shrimp processing industry is one of the major food processing industries in India and significant amount of waste is generated by this industry because of the large percentage of shrimp heads, exoskeleton, and soluble components not used during the various processing operations, which can be estimated around 1,25,000 to 1,50,000 ton per annum in Indian shrimp processing industries (Ramyaadevi *et al.*, 2012).

#### **CHEMICAL COMPOSITION OF SHRIMP WASTE**

The chemical composition of shrimp shell waste will vary significantly depending upon the nature of the processing operation. The most common methods of processing are hand-deheading and mechanical-peeling. The waste produced from each process varies in the level of chitin, protein, non-protein nitrogen, and calcium carbonate. The chemical composition of shrimp waste as reported by various authors is presented in Table 1.

## **AVAILABILITY OF SHRIMP SHELL WASTE**

The shrimp processing industry has been rapidly growing in India and Gopakumar (2002) has reported that the availability of shrimp processing waste in India is 1,00,000 tons per annum.

Mathew and Nair (2006) reported that shrimp processing waste generated in our country is around 1,00,000 tonnes per annum. Ramyadevi *et al.* (2012) reported that the processing waste generated in Indian shrimp processing industries would be around 1,25,000 to 1,50,000 ton per annum. Gortari *et al.* (2013) reported that the annual worldwide production of chitin is approximately  $10^{10}$  to  $10^{12}$  ton per annum

## **EXTRACTION AND CHARACTERIZATION OF CHITOSAN**

The main commercial process for chitosan extraction from shrimp shell waste is based upon demineralization by acid treatment, deproteinization by alkali treatment and deacetylation again by strong alkali. Islam *et al.* (2011) reported 15.21% chitosan yield from shrimp processing waste. Puvvada *et al.* (2012) reported 34% chitosan yield from crude chitin collected from exoskeleton of shrimp by chemical method. Selimot *et al.* (2013) also reported 14.7% chitosan yield from raw shrimp shells. Kamala *et al.* (2013) observed that the yield of chitin was 32% in the total weight of the dried shrimp shells and the yield of chitosan was 54.31% from chitin and infrared spectroscopy of the structure changes of initial chitin and chitosan were confirmed by FTIR spectroscopy with standard chitin and chitosan. Ameh *et al.* (2015) reported 15-20% yield of chitosan from shrimps by acid demineralization. Arafat *et al.* (2015) observed the yield of 19.0% chitosan from shrimp shell waste. Chitosan which was obtained by deacetylation of chitin can be characterized by using FTIR.

## **ROLE OF CHITOSAN IN IMPROVING PERFORMANCE, DIGESTIBILITY AND INTESTINAL MORPHOLOGY OF PIGS**

The small intestine is the main place for digestion and absorption of nutrients, and the intestinal mucosa plays an important role in these processes. Weaning stress may lead to a reduction in villus height and an increase in crypt depth (Pluske *et al.*, 1996) decreasing the surface area for nutrient absorption and thereby poor nutrient absorption and reduced performance (Pluske *et al.*, 1995). The crypt is the area where stem cells divide to permit the renewal of the villus, and a large crypt indicates fast tissue turnover and a high demand for new tissue. The ratio of villus height to crypt depth is a useful criterion for estimating the digestive capacity in the small intestine (Montagne *et al.*, 2003). The villus: crypt ratio is a single measure for evaluation of small intestine maturity and health in swine rather than villus

height or crypt depth Masri *et al.* (2015). Several authors have reported that chitosan of shrimp waste had beneficial effect on intestinal morphological structure.

For instance, Liu *et al.* (2008b) reported that dietary supplementation with 0.01 or 0.02% of COS positively affected FI, BWG, and FCR, increased the digestibility of nutrients, and enhanced villus height and the villus:crypt ratio in the ilea and jejunum of weaned pigs. Tang *et al.* (2005) demonstrated that the growth-promoting properties of chitosan may be related to increased blood concentrations of growth hormones and insulin-like growth factor 1 in the early-weaned piglets fed a diet containing COS (0.025%). Han *et al.* (2007a) found no influence of dietary COS on the BWGs of young pigs; however, compared to control pigs, animals fed a diet supplemented with COS (0.30 and 0.40%) exhibited better FCR and improved digestibility of dry matter and crude fat. Zhou *et al.* (2012) reported supplementation of COS (0.10 or 0.20%) improves growth performance, digestibility of nutrients in weaned pigs. They reported improved growth performance and total tract apparent digestibility of dry matter and nitrogen, as well as decreased incidence of diarrhea, as results of a higher inclusion level of COS (0.20%), but both performance and digestibility were lower than in pigs fed a diet supplemented with antibiotics (Zhou *et al.*, 2012). Yang *et al.* (2012) evaluated the effect of dietary COS (0.02, 0.04, or 0.06%) on the growth performance and intestinal morphology of weaned pigs. Pigs fed a diet containing 0.04 or 0.06% COS had improved BWG and FCR from day 1 to 14 postweaning, whereas dietary COS did not affect villous height or crypt depth of the duodenum, jejunum, or ileum. In contrast, Yan and Kim (2011) did not observe any effect of COS (0.30% in the diet) on growth performance and digestibility of nutrients.

Xu *et al.* (2013b) evaluated the effects of chitosan (0.01, 0.05, 0.10 or 0.20%) on growth performance, small-intestinal morphological structure, and concentrations of growth hormone (GH) in the serum and of weaned pigs, in order to study the regulating action of COS on pigs growth through endocrine and intestinal morphological approaches. BWG was improved quadratically by dietary COS; as well, chitosan tended to quadratically increase levels of serum GH and villus heights of the jejunum and ileum, as well as the ratio of villus height to crypt depth in the duodenum, jejunum and ileum. An optimal response was obtained at the 0.05% dietary level of COS. They indicated that the mechanism of improvement of growth performance by dietary COS may be due to increased GH concentrations in the serum and an ameliorated small-intestine morphological structure (Xu *et al.*, 2013b). In another experiment they found that COS (0.01-0.20% in the diet) increased the relative weight and length of the

duodenum as well as the length of the jejunum in weaned piglets (Xu *et al.*, 2013a). The results of their recent experiment (Xu *et al.*, 2014) confirmed the beneficial influence of dietary COS (0.01-0.20%) on growth performance of weaned pigs and indicated that this growth-promoting effect may also be due to improved digestibility of dry matter, crude protein, calcium and phosphorus, as well as increased levels of amylase in the jejunum of weaners fed a diet containing COS. Chen *et al.* (2009) found that increasing diet supplementation with COS (0.25, 0.50%) tended to linearly improve BWG, as well as apparent dry-matter and N digestibility in weaned pigs.

Han *et al.* (2007b) demonstrated no effect of COS (0.02-0.10% in the diet) on BWG, apparent nutrient digestibility, or the results of slaughter analysis; however, FCR was improved in pigs fed diets containing 0.05-0.10% COS. Dietary COS also had a beneficial effect on certain measurements of meat quality, i.e., drip loss and pork colour, as well as reduced LDL content in meat lipids (Han *et al.*, 2007b). The aim of a study by Wang *et al.* (2009) was to compare the effects of diet supplementation with COS (0.50%) or with tylosin on growth performance and nutrient digestibility in growing pigs. COS had no influence on BWG and FCR, while the digestibility of dry matter, nitrogen, and gross energy were significantly increased in pigs fed a diet supplemented with either COS or tylosin (Wang *et al.*, 2009). The results of the study with growing pigs (Yin *et al.*, 2010) demonstrated that dietary COS (0.01%) enhances the net absorption of many dietary amino acids into the portal vein, so it is effective in increasing the efficiency of dietary protein utilisation by pigs.

Swiatkiewicz *et al.* (2015) conducted experiments to determine the effects of chitosan on small intestinal morphological structure of piglets, in order to evaluate the regulating action of chitosan on weaned pig growth through endocrine and intestinal morphological approaches and reported chitosan increased quadratically the ratio of villus height to crypt depth in duodenum, jejunum and ileum on day 14 ( $p < 0.05$ ,  $p < 0.01$ ) and 25 ( $p < 0.01$ ), however, it decreased quadratically crypt depth in ileum on day 14 ( $p < 0.05$ ) and that in duodenum, jejunum and ileum on day 28 ( $p < 0.01$ ,  $p < 0.05$ ). They concluded that chitosan could quadratically improve growth in weaned pigs, and the underlying mechanism may be due to the increase of the serum GH concentration and improvement of the small intestine morphological structure.

## **ROLE OF CHITOSAN ON IMMUNE RESPONSE AND ANTIBACTERIAL EFFECTS**

The immunomodulatory properties of dietary COS (0.025%) were studied in early-weaned pigs by Yin et al. (2008). They reported that weaning stress resulted in decreased serum antibody and cytokine levels, while dietary COS increased IL-1 $\beta$  gene expression in jejunal mucosa and lymph nodes, as well as serum concentrations of IL-1 $\beta$ , IL-2, IL-6, IgA, IgG and IgM. The authors indicated that dietary COS may enhance the cell-mediated immune response in early-weaned piglets by modulating the production of cytokines and antibodies.

The objective of study by Yang et al. (2012) was to evaluate the effect of dietary COS (0.02, 0.04, 0.06%) on cecal microflora of weaned pigs. Pigs fed a diet containing 0.04% had increased concentrations of *Bifidobacteria* and *Lactobacilli* in the caecum and decreased concentrations of cecal *Staphylococcus aureus* on the seventh day after weaning, whereas 0.06% level of dietary COS increased the number of *Bifidobacteria* on fourteenth day postweaning (Yang et al., 2012). The authors gave two possible explanations for the observed antimicrobial activity of COS. One was that the positive charge on the NH<sub>3</sub><sup>+</sup> group of the COS glucosamine monomer causes interactions with negatively charged microbial cell membranes, leading to leakage of intracellular constituents; another is that it exerts an indirect influence through enhancing populations of *Bifidobacteria* and *Lactobacilli* and their subsequent competitive exclusion of *S. aureus* (Yang et al., 2012).

The aim of a study with non-challenged vs *Escherichia coli* lipopolysaccharide-challenged weaned pigs was to evaluate the effects of diet supplementation with COS (0.50%) on immune response indicators (Chen et al., 2009). In the post-challenge period, dietary COS decreased rectal temperature, reduced cortisol blood concentration, and increased IGF-1 concentration, but did not affect lymphocyte count. The authors concluded that dietary COS had little modulating influence on the inflammatory stress markers in weanling pigs (Chen et al., 2009).

The goal of an experiment by Liu et al. (2010) was to evaluate whether COS (0.016% in the diet) can replace an antibiotic (cyadox) in reducing signs associated with infection in weaned pigs challenged with *Escherichia coli*. The obtained data did not prove the efficacy of COS as a substitute for an antibiotic growth promoter for weaners infected with *E. coli*. In challenged pigs COS increased plasma IGF-I concentrations, decreased IgA-positive cell numbers in the jejunal and ileal lamina propria, reduced the incidence of diarrhea, and alleviated some other signs associated with infection; however, unlike cyadox, it did not alleviate the detrimental

effect of the challenge on growth performance (Liu et al., 2010). The results of a more recent study by Xiao et al. (2013) demonstrated, however, that dietary COS (0.03%) and feed antibiotic (chlortetracycline) have similar beneficial effects in reducing intestinal inflammation and promoting growth in weaned piglets challenged with enterotoxigenic *Escherichia coli*, i.e., a type of bacteria which often causes post-weaning diarrhea. Feeding with COS increased, similarly to antibiotic, concentrations of intraepithelial lymphocytes, increased villus length, villus length/crypt depth, and goblet cells, increased occludin protein and secretory IgA protein expressions, decreased Toll-like receptor 4 (TLR4) mRNA expression, and improved FCR; hence the authors concluded that chitosan has the potential to replace chlortetracycline as a feed additive for piglets (Xiao et al., 2013). Results of 70 d experiment with growing pigs (31 kg of initial body weight) demonstrated that dietary COS (0.10 or 0.30%) improved immune response (antibody titres) after vaccination with *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* (Han et al., 2007b).

Liu et al. (2008b) reported that dietary supplementation with 0.01 or 0.02% of COS reduced the incidence of diarrhea and *E. coli* counts, whereas increased *Lactobacillus* counts in the feces in weanling pigs. Han et al. (2007a) observed inhibited growth of harmful bacteria, measured as fecal *E. Coli* and *Clostridium spp.* counts, in young pigs (25 kg of body weight) fed diet supplemented with much COS dietary level (0.30 and 0.40%). Similarly, Yan and Kim (2011) found reduced fecal *Escherichia coli* counts in weaned pigs fed diet supplemented with 0.30% COS and the similar results were obtained by Wang et al. (2009) who reported that dietary COS (0.50%) reduced the number of fecal *Escherichia coli* in growing pigs, whereas the count of fecal *Lactobacilli* was unaffected.

In a completely randomised design, the effect of antibiotic or chitosan from shrimp waste as prebiotic was evaluated using 36 weaned piglets (42 days) by Yugandharkumar et al (2017). The standard grower ration (T1) was supplemented with chlorotetracyclin (T2) while shrimp shell meal was included at 2.5% (T3), 5.0% (T4) and 7.5% (T5), as a source of chitosan. The feed and water was made ad libitum. There was a significant increase ( $P < 0.01$ ) in serum total protein, primarily due to improvement in the globulin concentration, decrease ( $P < 0.01$ ) in serum total cholesterol, serum triglycerides, LDL Cholesterol and increase in HDL cholesterol due to shrimp waste supplementation and there was no significant difference among the pigs fed different treatments for the various carcass characters.

**ROLE ON HAEMATOLOGICAL CHARACTERS, LIPID METABOLISM, PORK QUALITY AND CARCASS CHARACTERS**

Results of the study with weanling pigs (Zhou et al., 2012) demonstrated that diet supplementation with 0.10 or 0.20% COS reduced blood lymphocyte concentration, without effect on erythrocyte and leukocyte concentrations. Yan and Kim (2011) reported no effect of COS (0.30% in the diet) on most hematological blood characteristics in weaned pigs, however, treatment with COS increased blood lymphocyte concentration. Results of the study with growing pigs (Wang et al., 2009) demonstrated that dietary COS (0.50%) had positive effect on blood biochemical profile, i.e., increased the serum HDL cholesterol concentration, without affecting other morphological and biochemical blood indicators, among others cholesterol or triglyceride concentration.

Kim *et al.* (2008) reported inclusion of COS in the diets of finishing pigs reduced the amount of cholesterol in pork without effecting the carcass characteristics and pork quality traits, such as dressing percentage, back fat thickness, lean percent, color and marbling score and drip losses. Two experiments were conducted to evaluate the effect of dietary lecithin with or without COS on the performance, pork cholesterol, fatty acid composition and quality of pork of finishing pigs and reported addition of COS in diets containing lecithin reduced pork cholesterol without effecting performance, carcass characters and pork quality.

Ma et al., 2001 reported that the mechanism in reduced triglycerides (TG) and total cholesterol in serum was COS can form a gel complex with gastric acid in the gastrointestinal tract where the gel complex cannot be degraded under the high pH environment in the intestine. This gel can absorb bile acid and cholesterol and the gel, bile acid, cholesterol mixtures are discharged in feces, thus the absorption of fat and cholesterol is decreased. Tang et al., 2005 reported supplementation of COS reduced ( $P < 0.05$ ) serum triglycerides and total cholesterol concentration and also suggested the reason for this response was that COS formed a gel complex with gastric acid in the gastrointestinal tract and was subsequently excreted in the feces.

**Table 1: Chemical composition and chitin (%) of shrimp shell meal (as reported by several authors )**

| CP   | EE   | TA   | CF   | Ca   | P    | Chitin | Reference                             |
|------|------|------|------|------|------|--------|---------------------------------------|
| 39.5 | 4.8  | 24.8 | 8.7  | 7.18 | 3.45 | 15.5   | Yugandhar Kumare <i>et al.</i> , 2017 |
| 32.6 | 1.7  | 0.3  | 24.9 | NA   | NA   | 14.7   | Selimot <i>et al.</i> , 2013          |
| 20.0 | 7.44 | 24.5 | 8.5  | NA   | NA   | NA     | Okonkwo <i>et al.</i> , 2012          |
| 68.7 | 6.8  | 10.5 | 0.4  | NA   | NA   | NA     | Ehigiator and Oteria, 2012            |
| 24.9 | 6.2  | 46.1 | NA   | NA   | NA   | 25.2   | Bellaaj <i>et al.</i> , 2012          |



|             |            |             |             |            |            |             |                                     |
|-------------|------------|-------------|-------------|------------|------------|-------------|-------------------------------------|
| 38.5        | 4.7        | 34.6        | 0.1         | NA         | NA         | NA          | Ehigiatoret <i>et al.</i> , 2011    |
| 49.0        | 4.8        | 27.0        | 18.2        | NA         | NA         | NA          | Sanchez-Camargo <i>et al.</i> 2011  |
| 36.6        | 10.2       | 21.7        | 19.5        | 4.9        | 1.2        | 18.9        | Khempakaet <i>et al.</i> , 2011     |
| 48.3        | 5.75       | NA          | 12.9        | 3.5        | 1.0        | NA          | Aktaret <i>et al.</i> , 2011        |
| 32.5        | 1.5        | 26.6        | 8.7         | NA         | NA         | NA          | Ravichandranet <i>et al.</i> , 2009 |
| 48.3        | 6.3        | 17.5        | 13.3        | NA         | NA         | NA          | Ingweyeet <i>et al.</i> , 2008      |
| 39.32       | 0.94       | 26.7        | 29.75       | 6.05       | 0.97       | 30.44       | Khempakaet <i>et al.</i> , 2006     |
| 46.3        | 9.04       | 17.0        | 4.3         | 7.0        | 3.03       | 9.82        | Okoyeet <i>et al.</i> , 2005        |
| 39.5        | 9.0        | 24.0        | 12.3        | 15.77      | 0.45       | NA          | Fanimoet <i>et al.</i> , 2004       |
| 35.0        | 3.0        | 30.0        | NA          | NA         | NA         | 15.0        | Gopakumar, 2002                     |
| 55.7        | 6.2        | 20.4        | 11.38       | 5.21       | 1.47       | NA          | Gernat, 2001                        |
| 44.0        | 7.3        | 22.8        | NA          | 10.5       | 1.2        | 18.1        | Ngoanet <i>et al.</i> , 2000        |
| 46.3        | 9.04       | 17.0        | 4.3         | 7.0        | 3.03       | 9.82        | Fanimo and Oduguva, 1999            |
| 50.89       | 6.31       | 15.6        | 8.92        | 5.21       | 1.47       | NA          | Rosenfeld <i>et al.</i> , 1997      |
| <b>40.6</b> | <b>5.2</b> | <b>20.9</b> | <b>11.9</b> | <b>7.5</b> | <b>1.5</b> | <b>16.7</b> | <b>Mean</b>                         |

NA: Not available

**Table 2: Effects of dietary COS (chitosan) supplementation in crossbred pigs**

| <b>Studied parameter</b>  | <b>Results</b>  | <b>Reference</b>          |
|---|---|---------------------------|
| Growth performance, FCR, serum biochemical indices, serum growth hormone and insulin-like growth factor-I levels in early-weaned piglets. | Improved growth and FCR by increasing plasma GH and IGF-I levels. Blood urea nitrogen level was reduced whereas serum total protein concentration was increased.                                    | Tang <i>et al.</i> , 2005 |
| Growth performance, nutrient digestibility, intestinal and fecal microflora in young pigs   | Improved FCR, increased digestibility of dry matter and crude fat, and decreased faecal <i>E. coli</i> and <i>Clostridium spp.</i> counts in pigs fed diet and no effect of dietary COS on the BWG. | Han <i>et al.</i> , 2007a |
| Growth performance, carcass quality, nutrient digestibility growing and finishing pigs  | Improved FCR in pigs fed diet with COS. No effect of dietary COS on BWG.  | Han <i>et al.</i> 2007b   |
| Growth performance, nutrient digestibility, blood metabolites and pork quality of finishing pigs  | Addition of COS in diets containing lecithin reduced pork cholesterol and did not affect performance nutrient digestibility, blood metabolites and pork quality.                                    | Kim <i>et al.</i> , 2008  |
| Growth performance,   | Increased FI, BWG, FCR, nutrient  | Liu <i>et al.</i> , 2008b |

|   |  |                   |
|---|--|-------------------|
| nutrient digestibility, fecalmicroflora, and intestinal morphology in weanling pigs                 | digestibility, villus height and the villus:crypt ratio in the ilea and jejunum, and <i>Lactobacillus</i> counts in the faeces, whereas reduced the incidence of diarrhea and <i>E. coli</i> counts in the faeces of pigs fed diet supplemented with COS.                          |                   |
| Immune response indices in weanling pigs  | Increased IL-1 $\beta$ gene expression in jejunal mucosa and lymph nodes, as well as serum concentrations of IL-1 $\beta$ , IL-2, IL-6, IgA, IgG and IgM in pigs fed diet supplemented with COS.   | Yin et al., 2008  |
| Growth performance, nutrient digestibility, blood indices, immune response indices in weanling pigs | Linear improve of BWG, as well as dry-matter and N digestibility by dietary COS. No effect of COS on blood indices. In the post-challenge period, decreased rectal temperature, reduced cortisol blood concentration, and increased IGF-1 concentration in pigs fed diet with COS. | Chen et al., 2009 |
| Growth performance, nutrient digestibility, blood indices, fecalmicroflora in growing pigs          | Improved digestibility of dry matter, nitrogen, and gross energy, increased serum HDL cholesterol concentration, and decreased the count of fecal <i>E. coli</i> , in pigs fed a diet supplemented with COS. No effect of dietary COS on BWG and FCR.                              | Wang et al. 2009  |
| Immune response in weanling pigs  | COS reduced the incidence of diarrhea and alleviated some other signs associated with infection; however, unlike antibiotic, it did not alleviate the detrimental effect of the challenge on growth performance  | Liu et al., 2010  |
| Absorption of amino acids in growing pigs   | Positive effect of dietary COS on the net absorption of amino acids  | Yin et al. 2010   |
| Growth performance, nutrient digestibility, blood indices, fecalmicroflora in weanling pigs         | No effect of dietary COS on growth performance, nutrient digestibility, and most blood indices. Increased blood lymphocyte concentration and reduced fecal <i>Escherichia coli</i> counts in weaned pigs fed diet supplemented with COS  | Yan and Kim, 2011 |
| Growth performance, intestinal morphology, cecalmicroflora in weanling pigs                         | Improved growth performance from d 1 to 7 postweaning and from d 1 to 14 postweaning. Increased concentrations of <i>Bifidobacteria</i> and <i>Lactobacilli</i> in the cecum and decreased number of cecal <i>Staphylococcus aureus</i> in pigs fed diet with COS.                 | Yang et al., 2012 |
| Growth performance, nutrient digestibility, diarrhea incidence, and                                 | Positive effect of dietary COS on growth performance and apparent digestibility of dry matter and nitrogen. Decreased  | Zhou et al., 2012 |

|   |   |                                      |
|---|---|--------------------------------------|
| blood indices in weanling pigs  | diarrheas incidence and blood lymphocyte concentration in pigs fed diet with COS.   |                                      |
| Growth performance, intestinal morphology structure, level of growth hormone (GH) in the serum in weanling pigs | Quadratically improved BWG by dietary COS. COS tended to increase of serum GH and villus heights of the jejunum and ileum, as well as the ratio of villus height to crypt depth in the duodenum, jejunum and ileum.   | Xu et al., 2013b                     |
| Intestinal mucosal barrier function in weanling pigs  | Similarly to antibiotic (chlortetracycline) COS increased intestinal expression of occludin protein, secretory immunoglobulin (sIgA) protein, and decreased Toll-like receptor 4 (TLR4) mRNA.   | Xiao et al. 2013                     |
| Growth performance, nutrient digestibility, activity of intestinal enzymes in weanling pigs                     | Dietary COS quadratically increased BWG, apparent digestibility of crude protein, dry matter, Ca, and P, as well as enhanced amylase activity of proximal jejunum.  | Xu et al. 2014                       |
| Gut microbiota and intestinal luminal metabolites in weaned mini piglets  | Dietary supplementation with COS modifies the composition of ileal and colonic microbiota and increased the beneficial intestinal bacteria.   | Kong et al., 2014                    |
| Growth performance and small intestinal morphological structure of piglets                                      | Average body weight gain of pigs was improved quadratically and increased quadratically the villus height of jejunum and ileum and increased quadratically the ratio of villus height to crypt depth in duodenum, jejunum and ileum.  | Swiatkiewicz et al., 2015            |
| Immune response, haematological characters and lipid profile in crossbred weaned piglets                        | Significant increase ( $P < 0.01$ ) in serum total protein, primarily due to improvement in the globulin concentration, decrease ( $P < 0.01$ ) in serum total cholesterol, serum triglycerides, LDL Cholesterol and increase in HDL cholesterol due to shrimp waste supplementation. | Yugandhar Kumar <i>et al.</i> , 2017 |

## Conclusion

Summarising the literature data presented in this review paper, it can be concluded that shrimp shell meal containing chitosan can be used as a feed additive for pigs has some beneficial immunomodulatory, antioxidative, antimicrobial, and hypocholesterolaemic properties. It ought to be stressed that, unlike many other kinds of feed additives, in most

research these properties of chitosan were reflected in improved growth performance (body weight gain and/or feed conversion ratio) and nutrients digestibility in pigs. Many authors, based on their experimental data, indicated that chitosan has a growth-promoting effect similar to that of feed antibiotics and can be used as an effective alternative to them.

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