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Review Article

Chitosan: a promising natural polysaccharide feed additive in poultry production systems

Abd El-Ghany, W. A.

Poultry Diseases Department, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt

Correspondence: *W. A. Abd El-Ghany, Poultry Diseases Department, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt. E-mail: wafaa.soliman@cu.edu.eg*

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Abstract

In recent years, the hazardous use of antibiotic growth promotors in the poultry industry has led to the development of drug resistance and violative tissue residues. Therefore, the European Union Regulation banned application of these growth promotors, and the international authorities have searched for other natural and safe feed additive sources as substitutes for antibiotics. Chitosan has been used as a feed-additive alternative in veterinary medicine practices worldwide. Chitosan and chitosan-based nanoparticles have been extensively investigated in the poultry production system and have proved several positive impacts. The overall performance parameters of broilers and layers have been improved following dietary treatments with chitosan. Besides, chitosan showed antimicrobial activity against many bacterial, fungal, viral, and parasitic diseases as well as boosting of the immune response. Modulation of the antioxidant activity and modification of some blood parameters have also been detected owing to dietary chitosan supplementations. Moreover, chitosan nanoparticles have been now applied as a vaccine delivery vehicle and a mucosal adjuvant for many important poultry bacterial and viral diseases. Therefore, this review article sheds light on the effects of chitosan and its nanoparticle forms on the production traits of broilers and layers, their antimicrobial, immuno-regulatory, and antioxidant properties, as well as their effects on the blood constituents and vaccine production.

Key words: Antioxidant and antimicrobial, Chitosan nanoparticles, Immunity, Poultry production trait, Vaccine

Introduction

The application of antibiotics as feed additives is prohibited as a result of the development of bacterial resistance, the presence of residues in animal products, and environmental pollution (Hu *et al*., 2018). Therefore, the European Union Regulation banned using these antibiotics as growth promotors in animal production (European Union Regulation, 2003), and the international authorities searched for natural and safe feed additive sources as substitutes to antibiotics. Dietary supplementations of poultry with probiotics, prebiotics, synbiotics, parabiotics, postbiotics, microalgae, and immunoglobulins preparations have been developed to improve the feed utilization efficiency and to maintain the general health conditions (Abd El-Ghany, 2020a, b, 2021; Abd El-Ghany *et al*., 2022a, b). The phytobiotics containing a large variety of plant-derived products such as essential oils, extracts, herbs, and oleoresin showed positive impacts on the host's productivity and the final product quality (Hady *et al*., 2016; Zaki *et al*., 2016; Abd El-Ghany, 2020c). Moreover, several types of these phytobiotics have been effectively used in the poultry industry as growth promoters, antimicrobials, and immuno-modulators (Abd El-Ghany and Eraky, 2019; Abd El-Ghany, 2020d; Abd El-Ghany, 2022; Abd El-Ghany and Babazadeh, 2022).

Chitosan has been approved by the Food and Drug Administration in 2001 in United States of America (Wang *et al*., 2020). Chitosan originates from alkaline deacetylation of chitin in the exoskeleton of shrimp, crabs, squid, insects, and fungal biomass (Tømmeraas *et al*., 2011). It is a natural biodegradable poly-aminosacharide (Vimal *et al*., 2013). The structure of chitosan particles is presented in Fig. 1. Chitosan is a cheap, renewable, non-toxic, compatible, and safe compound with no side effects, tissue residues, or resistance (Huang *et al*., 2015). There are wide ranges of chitosan applications in the agricultural, food science, textile, pharmaceutical, and biomedical fields (Naskar *et al*., 2019). Moreover, chitosan can act as an adjuvant for vaccines and drugs delivery (Zhao *et al*., 2017) due to its ability to carry and deliver compounds through the different administration routes.

In the field of veterinary medicine, chitosan has been extensively used for livestock as a feed-additive alternative for antibiotics due to its multiple and beneficial bioactivities (Anraku *et al*., 2018; Darwesh *et*

al., 2018; Ravi *et al*., 2018). Dietary chitosan plays important roles in improving the overall growth parameters and gut microflora, modulating the immune response, enhancing the antimicrobial, antioxidant, and anti-stress activities (Ma *et al*., 2017; Li and Zhuang, 2020; Osho and Adeola, 2020). The hypo-lipidemic and anti-cancer effects of chitosan have also been reported (Zhang *et al*., 2013).

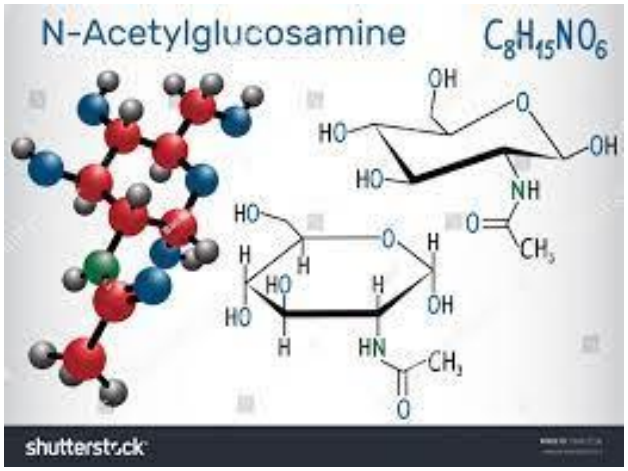

Fig. 1: The structure of chitosan particles

Nanotechnology has become important in diagnosing and preventing many diseases in veterinary medicine (Gopi *et al*., 2017). Chitosan-based nanoparticles have attracted considerable attention because of their inherent biocompatibility and biodegradability and lack of toxicity (Li *et al*., 2018). They have been shown to be effective carriers for antigen delivery (Imam *et al*., 2021). Chitosan in nanoparticle forms can improve the mucosal adhesion, permeability, stability, extended antigen release at the mucosal sites, and increased bioavailability (Mohajer *et al*., 2014). Besides, chitosan-nanoparticle-based vaccines have been extensively applied in poultry production to reduce infections with *Salmonella enteritidis* (Acevedo-Villanueva *et al*., 2021a, 2022), *Campylobacter jejuni* (Singh *et al*., 2019), *Escherichia coli* (Kaikabo *et al*., 2017), *Clostridium perfringens* (Akerele *et al*., 2020a), Newcastle disease virus (NDV) (Zhao *et al*., 2018), avian influenza virus (AIV) (Hajam *et al*., 2020), and infectious bronchitis virus (IBV) (Lopes *et al*., 2018).

In this respect, this review article sheds light on the different effects of chitosan and its nanoparticle forms on the production traits of broilers and layers, their antimicrobial, immuno-regulatory, and antioxidant properties, as well as their effects on the blood constituents and vaccine production.

The different effects of chitosan on poultry production system

Production traits

The beneficial effects of chitosan on the growth performance parameters, including body weight (BW), BW gain (BWG), feed intake (FI), and feed conversion ratio (FCR), were documented in broiler chickens (Zhou *et al*., 2009; Pramujo *et al*., 2019), especially when birds fed on chitosan diet from the age of a day-old (Table 1). Chitosan can improve growth parameters via different modes of action; it may help in the establishment of beneficial intestinal microflora and consequently, improvement of digestion and absorption of nutrients (Shi *et al*., 2005; Ravi *et al*., 2018), increase the ileal digestibility of nutrients (Huang *et al*., 2005), and improve the intestinal architecture with hypertrophied intestinal villi and epithelial cells (Khambualai *et al*., 2009). The action of chitosan may persist for a long time due to its slow motility in the highly viscus gut (Osho and Adeola, 2019). In addition, stimulation of digestive enzymes secretion from the stomach, pancreas, and intestinal walls, as well as increasing the levels of growth hormones and insulin-like growth factor I in the serum, were recorded following feeding on chitosan (Le *et al*., 2015).

It has been demonstrated that chitosan administration may improve carcass characteristics due to enhancement of growth performance parameters. The effects of chitosan on the carcass trait parameters have been investigated in many spp. of poultry with variable results (Table 2).

In layers, the quantity and quality of eggs could be affected by the dietary addition of chitosan (Table 3). Most of the previously conducted studies showed that chitosan and its derivatives have positive influences on egg weight, yolk colour, and composition, cholesterol content, etc. (Meng *et al*., 2010; Swiatkiewicz *et al*., 2013; Hernawan *et al*., 2017; Farivar *et al*., 2018).

Antimicrobial effect

The antimicrobial activity of chitosan has been previously reported (Ma *et al*., 2017). Chitosan has bactericidal and bacteriostatic properties (Goy *et al*., 2009), therefore, it is a good alternative for antibiotics. Chitosan showed an improvement in gut function and microbial populations (Nuengjamnong and Angkanaporn, 2018). Moreover, it could inhibit the activity of some Gram-positive and Gram-negative bacteria and fungi (Li and Zhuang, 2020). It has been reported that chitosan can probably reduce the permeability of bacterial cell membranes through the interaction between positively charged amino groups of chitosan and negatively charged bacterial cell membranes (Kong *et al*., 2010; Menconi *et al*., 2014). Sebti *et al*. (2005) related the antimicrobial potential of chitosan to its ability to penetrate the microorganism's nuclei, binding with the microbial DNA and consequently inhibiting the synthesis of mRNA and protein. Besides, the protonated chitosan can cover the bacterial cell surface, prevent the extravasation of cell contents, make the positive charge cells repel each other, and thus inhibit agglutination (Lim and Hudson, 2004).

Dietary chitosan inoculation increased the gut's populations of beneficial bacteria such as *Lactobacillus* spp., but inhibited pathogenic *E. coli* and *Salmonella*

spp. (Alishahi, 2014; Hassan *et al*., 2021). Likewise, Li *et al*. (2007) showed that adding 100 mg of chito-oligosaccharide to the broiler chicken diets reduces the cecal count of *E. coli*. Further, Tufan *et al*. (2015) demonstrated that the intestinal *E. coli* level was significantly decreased when quails were fed on a diet containing 150 mg chitosan-oligosaccharides/kg. In the same study, quail diets containing 75 or 150 mg/kg of chitosan-oligosaccharides resulted in reduced *Lactobacillus* spp. count in the gut (Tufan *et al*., 2015).

Table 1: The effects of chitosan and its derivatives on production traits of broilers

Chitosan type	Dose/diet	Effects	Reference
Chitosan	30 g/kg	↓ BW, and daily FI Poor FCR	Razdan and Pettersson (1996)
Chitosan and flavomycin	0.005, 0.010, and 0.015%	↑ BWG, and the ileal digestibility of dry matter, Ca, P, crude protein, and amino acids Improved FCR	Huang *et al*. (2005)
Chitosan	0.05-0.1%	↑ BWG and improved FCR and nitrogen retention for a diet containing 0.1% chitosan No significant effect on BWG, AFI, and FCR for a diet containing 0.05% chitosan	Shi *et al*. (2005)
Copper chelates chitosan	100 ppm	↑ BWG and dry matter digestibility during the starter period of rearing	Lim *et al*. (2006)
Chito-oligosaccharide	0.005% or 0.01%	↑ AFI, digestibility of dry matter, energy, calcium, and phosphorus, and concentrations of cecal *Lactobacillus* microbial flora ↓ cecal *E. coli*	Li *et al*. (2007)
Chitosan	200 mg/kg	↑ growth performance, growth hormones, and insulin-like growth factor I in serum	Jin (2008)
Chitosan	0.06%	↑ AFI and BWG	Khambualai *et al*. (2009)
Chitosan oligosaccharide and/or beta-glucan + organic zinc	0.025%	No effect the growth parameters	Keser *et al*. (2012)
Chitosan	0.01%	↑ growth indices, nutrients digestibility, and retention of N and Ca	Swiatkiewicz *et al*. (2014)
Chitosan	2 g/kg	Improved FCR	Nuengjamnong and Angkanaporn (2018)
Chito-oligosaccharide	30 mg/kg	↑ AFI, digestibility of dry matter, energy, calcium, and phosphorus, and concentrations of caecal *Lactobacillus* microbial flora ↓ cecal *E. coli*	Li *et al*. (2019)
Chitosan	1.0 g/kg	↑ BWG	Osho and Adeola (2019)
Chitosan Nano-chitosan	50 and 70 mg/kg 30 and 50 mg/kg	↑ BW and BWG of quails	Hassan *et al*. (2021)

BW: Body weight, FI: Feed intake, FCR: Fed conversion ratio, and AFI: Average feed intake

Table 2: The effects of chitosan and its derivatives on carcass characteristics of chickens, quails, and geese

Chitosan type	Dose/diet	Effects	Reference
Chitosan	50 or 100 mg/kg	↑ carcass ratio and leg and wing ratio ↓ carcass-liver ratio	Tufan and Arslan (2012)
Chitosan	75 or 150 mg/kg	No effect on the carcass weight, and heart, liver, and gizzard percentages in Japanese quail	Tufan *et al*. (2015)
Chitosan and neem leaf meal	0.025% and 0.05%	↓ abdominal fat	Sirsat Shraddha *et al*. (2017)
Chitosan oligosaccharides and L-carnitine individually or concurrent	100 mg/kg	No significant effect on carcass weight, carcass ratio, and heart, spleen, and gizzard ratio to carcass weight	Arslan and Tufan (2018)
Cricket chitin and cricket chitosan Shrimp chitin and shrimp chitosan	0.5 g/kg 0.5 g/kg	No positive influence on the carcass and organ characteristics	Lokman *et al*. (2019)
Chitosan	200 mg/kg	No significant positive effects on the dressing percentage, eviscerated carcass percentage, and halt-eviscerated carcass percentage in Huoyan geese	Miao *et al*. (2020)
Chitosan Nano-chitosan	50 and 70 mg/kg 30 and 50 mg/kg	No significant effect on dressing %, liver %, heart %, and total edible parts % in quails	Hassan *et al*. (2021)

Table 3: The effects of chitosan and its derivatives on production traits of layers

Chitosan type	Dose/diet	Effects	Reference
Chitosan	0.4 gm/kg of BW/day	↓ cholesterol, triglycerol, and free fatty acids in serum	Hirano *et al*. (1990)
Chitosan and shark cartilage	20 or 30 g/kg	↓ cholesterol, and palmitic and stearic acids in yolk ↑ oleic acid in a group given 30 g chitosan No effect on eggs weights and eggs component weights	Nogueira *et al*. (2003)
Chitosan and mineral complex	1%, 2%, or 3%	↑ haugh unit	Yoo *et al*. (2006)
Chito-oligosaccharides	0.02% or 0.04%	↑ laying egg rate, average egg weight, haugh units, and apparent digestibility of dry matter and nitrogen	Meng *et al*. (2010)
Chito-oligosaccharides and delta-amino levulinic acid	0.01% or 0.02%	↑ egg weight, yolk color, and haugh units Egg production and the egg-shell quality indices were not affected	Yan *et al*. (2010)
Chitosan and 20% distillers dried grains	0.01%	↑ hen-day egg production, daily egg mass, nutrient digestibility, and nitrogen and calcium deposition ↓ cholesterol content in yolk	Swiatkiewicz *et al*. (2013)
Chitosan	150 ppm/g	↓ cholesterol and malondialdehyde blood levels	Hernawan *et al*. (2017)
High degree of deacetylated chitosan	200, 400, 800 and 1600 ppm	↓ oxidation ability of egg yolk ↑ antioxidant performance of plasma	Farivar *et al*. (2018)
Chitosan	100 mg/kg	↑ fracture strength, bending load, and mineralization of femur	Swiatkiewicz *et al*. (2018)
Chitosan nanoparticles	200 mg/kg	↑ egg quality, egg yolk composition, immunity, and beneficial intestinal bacteria	Hamady and Farroh (2020)

Chitosan at concentrations of 1 and 2 g/kg diet enhanced the number of microflora such as *Bacillus* spp., but reduced the *E. coli* population (Nuengjamnong and Angkanaporn, 2018). Moreover, the clinical signs, including diarrhea, apathy, and ruffled feathers, as well as the pathological changes in *Salmonella gallinarum* infected broilers, were reduced following feeding on a diet with 3% chitosan, while the BWG was increased (Balicka-Ramisz *et al*., 2007). The results of a study by Menconi *et al*. (2014) indicated that the dietary inoculation of chitosan (0.2%) significantly reduces the colony-forming units of *Salmonella enterica* serovar Typhimurium in the crop, caecum, and the carcass of the experimentally infected broilers chicks.

It has been shown that nano-chitosan exhibits higher antibacterial activity than chitosan (Shaltout *et al*., 2019). The study by Levi Enoka *et al*. (2021) demonstrated that garlic and onion extract chitosan nanoparticles enhanced the presence of commensal beneficial gut bacteria such as *L. acidophilus* and reduced the number of pathogenic *E. coli* and *C. jejuni* in chickens to acceptable levels. Diet containing *Rhizopus stolonife* chitosan and nano-chitosan at 100 or 200 mg/kg body weight, respectively, resulted in a strong antimicrobial activity without toxicity (Darwesh *et al*., 2018). It is important to mention that chitosan nanoparticles also show antifungal property. For instance, Abdeltwab *et al*. (2019) demonstrated that nano-chitosan at concentrations between 3.0 and 4.5 µg/ml powerfully delayed the fungal spoilage effect.

Recently, the antiviral activity of chitosan nano-particles has been evaluated. Chitosan activates the production of interferon (IFN) genes, which support the production and regulation of innate and adaptive antiviral actions (Wani *et al*., 2014). Ebrahimi *et al*. (2019) found that intramuscular vaccination with inactivated infectious bursal disease virus (IBDV) vaccine along with chitosan solution (1% and 0.5%) significantly increases the specific humoral immune response induced by the vaccine. However, the higher antibody responses were obtained with a concentration of 1% chitosan. Recently, Elmasry *et al*. (2022) showed that copper chitosan-nanocomposite enhances immune expression and adaptive immunity, decreasing in the organs viral load in broilers with infectious anaemia. Moreover, the Egyptian study of Nasef *et al*. (2022) revealed that iron oxide chitosan-nanocomposite significantly decreases IBDV load in the bursa of Fabricius and ameliorates the pathological lesions in lymphoid organs. Regarding the parasitic infestation of birds, a daily dose of chitosan (0.6 g/bird) inhibited the development of coccidiosis in broiler chickens and improved the immunization programs in poultry production (Balicka-Ramisz *et al*., 2008).

Immune regulation

Chitosan can potentially promote the immune function and improve the antibody titers in the poultry serum. The different effects of chitosan on the immune response of broilers vary according to the species and ages of birds and the molecular weights, dosages, and duration of chitosan supplementation (Chi *et al*., 2017). Numerous studies have demonstrated that chitosan is an effective and safe adsorption enhancer that improve both the humoral and the cell-mediated immune responses (Yuan and Chen, 2012; Volkova *et al*., 2014). In growing ducks, Yuan and Chen (2012) reported that diet supplementations with 0.12% or 0.24% chitosan increased the weight of immune organs and lymphocyte proliferation. Besides, the dietary addition of 0.005%,

0.010%, and 0.015% chitosan-oligosaccharides increased the concentrations of the circulating immunoglobulin (IgG), IgA, and IgM, as well as enhanced the weight of bursa of Fabricius and thymus in 21-day-old broilers (Huang *et al*., 2007). A concentration of 0.01% chito-oligosaccharide in the diet of broiler chickens showed distinct effects on the immune functions in terms of increasing the weight of spleen, thymus, and bursa of Fabricius, IgM production, optimizing macrophage function by stimulating the release of cytokines [tumor necrosis factor α (TNF-α), interleukin (IL-1b and IL-6) and IFN-c], and activating inducible nitric oxide synthase to produce nitric oxide (Deng *et al*., 2008; Li, 2009). Similarly, adding chitosan at 200 mg/kg of diet increased the serum level of IL-2 and TNF-α in growing Huoyan geese (Miao *et al*., 2020). Chitosan oligosaccharides in a concentration of 350 mg/kg diet increased the relative weights of thymus, bursa of Fabricius, and spleen, as well as the percentages of G2/M phase thymocytes in 42-day-old broiler chickens (Chi *et al*., 2017). On the other hand, dietary inclusion of 50 mg/kg chitosan oligosaccharides significantly reduced the relative weight of spleen in broiler chickens (Wang *et al*., 2013).

The amino groups in chitosan could be recognized by the host's immune system, stimulate the production of serum antibodies, activate macrophages and natural killer cells, and in sequence improve the immune response (Li *et al*., 2015). Also, the dietary supplementation of broiler chickens with copper chitosan nanoparticles increased the levels of lysozyme, immune organ indexes, immunoglobulins, and some of the complement system proteins (Wang *et al*., 2011). Similarly, copper chitosan nanocomposite improved the innate immunity (phagocytic activity, lysozyme, and nitric oxide) as well as the cytokines levels (mRNA of IFN-γ and IL-6 and IL-10) in broiler chickens (Elmasry *et al*., 2022). Moreover, Choi *et al*. (2016) demonstrated that chitosan may be considered an immune-modulating adjuvant for T-cells and antigen-presenting cells in case of herpes simplex virus type 1 infection. The toll-like receptor 4 mediates the stimulating activities of chitosan oligosaccharide on macrophages (Zhang *et al*., 2014). Moreover, chitosan can modulate the functional activity of the antigen presenting cells. It could be taken up by macrophages, triggers inflammatory signal transduction, and activates the expression of cytokines and production of type I IFN (Fong and Hoemann, 2018).

Antioxidant properties and modifications of some blood parameters

Chitosan showed a strong antioxidant property in reducing glutathione peroxidase and catalase activities. However, it increased the malondialdehyde level in the hepatic cells, including the animal species or *in vitro* model. The studies discussed the effects of chitosan and its derivatives on the oxidative status of birds are demonstrated in Table 4. The antioxidant effect of chitosan may be attributed to its reaction with free radicals such as active hydroxyl and amino groups of its chain. Both groups could be used as hydrogen donors to the proxy unstable free radicals. Chitosan and its derivatives can scavenge hydroxyl radicals and superoxide anions free radicals, thus protecting the cells from damage (Sun *et al*., 2007) and mitigating oxidative stress (Swiatkiewicz *et al*., 2015).

Changes in different blood parameters after treatment of birds with chitosan are shown in Table 5. The dietary inclusion of 0.3% chitosan in broiler chickens diet reduced the ileal fat digestibility and plasma cholesterol level, but improved the ratio of high-density lipoprotein to total cholesterol (Razdan and Pettersson, 1996). Moreover, chitosan increased the gastric and duodenal viscosities and the binding of duodenal micelle components, consequently delayed the gastric emptying (Razdan and Pettersson, 1996). The hypo-cholesterolemic effect of dietary chitosan is correlated with its ability to bind bile acids and, consequently reduce duodenal bile acids concentrations (Razdan *et al*., 1997). The amount of dietary chitosan and the degree of deacetylation can affect the plasma lipids and protein profile (Yao and Chian, 2002). Chitosan has shown a hypocholesterolemic effect (Xia *et al*., 2011). Plasma triglycerides are produced from triglyceride-rich lipoproteins in the liver. Accordingly, diminishing lipogenesis in the liver results in reducing the level of triglycerides in the blood (Zhou *et al*., 2009). The dietary supplementation with chitosan and nano-chitosan

Table 4: The effect of chitosan and its derivatives on the oxidative status of chickens and quails

Chitosan type	Dose/diet	Effects	Reference
Chitosan	0.05-0.10%	↑ nitric oxide content, inducible nitric oxide activity in serum, and intestinal inducible nitric oxide mRNA expression	Li *et al*. (2009)
Chito-oligosaccharide	200, 350, and 500 mg/kg	↑ capacity and inhibit hydroxy radical and glutathione, S and gap 2/mitosis (G2M) phases, and proliferating index of ileum mucosal lymphocytes ↓ malonedialdehyde	Li *et al*. (2017)
Chito-oligosaccharide	30 mg/kg	↑ ileal mucosa antioxidant enzyme activity	Li *et al*. (2019)
Chitosan-oligosaccharide	2 g/kg	↑ antioxidative function	Osho and Adeola (2020)
Chitosan Nano-chitosan	50 and 70 mg/kg 30 and 50 mg/kg	↑ total antioxidant capacity and catalase enzyme activity of quails	Hassan *et al*. (2021)

Table 5: The effect of chitosan and its derivatives on some blood parameters of chickens and quails

Chitosan type	Dose/diet	Effects	Reference
Chitosan	0.4 gm/kg of BW/day	↓ cholesterol, triglycerides, and free fatty acids in serum	Hirano *et al*. (1990)
Chitosan	0.01%	↑ HDL and cholesterol in serum, ↓ triglyceride and total cholesterol concentrations	Li *et al*. (2007)
Chitosan	0.14% or 0.28%	No effect on the serum total protein and albumin ↑ blood cell count, and HDL and cholesterol blood concentrations ↓ saturated fatty acids and triglycerides concentration ↑ total protein blood concentration	Zhou *et al*. (2009)
Chitosan	0.02, or 0.04%	↑ white blood cells and total protein blood concentrations	Meng *et al*. (2010)
Chito-oligosaccharide and delta-amino levulinic acid	0.01%, or 0.02%	↑ concentrations of red and white blood cells and lymphocytes	Yan *et al*. (2010)
Chitosan and/or beta-glucan and organic zinc	0.025%	↓ LDL No effect on total cholesterol, HDL, and triglycerides	Keser *et al*. (2012)
Chitosan	100 mg	No effect on the total serum protein and albumin concentrations	Arslan and Tufan (2018)
Chitosan	1 and 2 g/kg	No influence on plasma triglycerides levels	Nuengjamnong and Angkanaporn (2018)
Chitosan Nano-chitosan	50 and 70 mg/kg 30 and 50 mg/kg	↑ HDL and triglycerides concentration ↑ total protein and albumin levels in quails	Hassan *et al*. (2021)

significantly decreases the total plasma cholesterol and the low density lipoprotein (LDL) concentrations, but increases the high density lipoprotein (HDL) concentration (Gallaher *et al*., 2000). The cholesterol-lowering effect of chitosan may be induced by decreasing cholesterol absorption from the gut, increasing the intestinal viscosity, and lowering the plasma and liver cholesterol (Gallaher *et al*., 2000). Moreover, chitosan may inhibit the pancreatic lipase activity, reducing the plasma cholesterol level. Kobayashi *et al*. (2002) found that dietary chitosan reduces the excessive amount of abdominal fat deposition and the lipase activity of the intestinal contents of broilers.

Vaccine delivery

Chitosan nanoparticles are now applied as a vaccine delivery vehicle and a mucosal adjuvant using different routes of inoculation (Malik *et al*., 2018). Chitosan has many advantages for vaccine production. For example, the positive charge of chitosan interacts with the negative charge of sialic acid in mucus (Illum *et al*., 2001), increasing the antigen mucosal absorption (Dyer *et al*., 2002). Moreover, chitosan is able to open the tight cell junctions and increases the permeability of antigens into cells (Kammona and Kiparissides, 2012). The biodegradability, biocompatibility, non-reactogenicity, low cost of production, immunomodulation (Zhao *et al*., 2017), as well as flexibility to perform modifications and conjugation with other polymers (Sosnik *et al*., 2014) have made chitosan a good adjuvant. Additionally, the number of positive charges of chitosan is reduced when exposed to pH 6.5, thus reducing its water solubility with a maximum delivery efficiency (Wu *et al*., 2016). However, the optimization of a preparation method is important to obtain the required size and surface charge of the vaccine. The application of chitosan and its derivatives in the delivery of different avian vaccines is shown in Table 6.

It has been reported that orally delivered chitosan nanoparticle-based vaccines can overcome the gastrointestinal antigen degradation problem by uptalking into Peyer's patches of poultry and pigs (van der Lubben *et al*., 2001). Moreover, intranasally delivered chitosan nanoparticle-based vaccines inhibited muco-ciliary clearance (Bernocchi *et al*., 2017) and reached nasal lymphoid tissues that have B-cells, T-cells, macrophages, and dendritic cells (Illum *et al*., 2001). Mucosal chitosan nanoparticles targeting the antigen-presenting cells may result in efficient antigen processing with induction of cell-mediated immunity and memory cells (Jabbal-Gill *et al*., 2012). The synthesized chitosan nanoparticles should have a high cationic charge, an average particle size of 500 nm for good distribution, 70% encapsulation efficacy for entrapped antigens, and 40% encapsulation efficacy for surface-conjugated antigens (Renu *et al*., 2018).

Chitosan nanoparticles were prepared as an adjuvant for vaccines for some important viral infections of poultry such as NDV (Zhao *et al*., 2012; Dai *et al*., 2015). Encapsulated N-2-hydroxypropyl trimethyl ammonium chloride chitosan (N-2-HACC)/NDV/IBV and N-2-HACC-N,O-carboxymethyl chitosan (CMC)/NDV-IBV were used as adjuvants for vaccines and the results revealed that intranasal immunization of chickens induced higher titers of IgG and IgA as well as IL-2, IL-4 and IFN-c than the combined attenuated live vaccine (Zhao *et al*., 2017). Moreover, O-2'-Hydroxypropyl trimethyl ammonium chloride chitosan (O-2'-HACC) was used as an adjuvant and mucosal immune delivery carrier for DNA vaccine for NDV *F* gene plasmid DNA and C3d6 molecular adjuvant (O-2'-HACC/pFDNA microparticles (Zhao *et al*., 2021)).

Table 6: Application of chitosan and its derivatives in the delivery of different avian vaccines

Disease	Type of vaccine	Birds species/route	Findings	Reference
Salmonellosis	Triple doses (500, 1.000, and 2.000 µg) of *S. enteritidis* in chitosan-nanoparticle	Broiler chickens/oral	↑ IgG and IgA antibodies, and IL-1β, IL-10, and IL-4 mRNA ↓ *S. heidelberg* in liver and spleen, and *S. enteritidis* load in cecum No significant effect on BWG or FCR	Acevedo-Villanueva *et al*. (2020)
	10 µg *S. enteritidis*, and 2 or 3 doses of nano-vaccine	Broiler chickens/oral	↑ mucosal, systemic, and cell mediated immune responses, and toll like receptor mRNA ↓ *S. enteritidis* presence in the cecum No significant effect on BWG or FCR	Han *et al*. (2020a)
	Double doses (12.5 and 50 µg) of *Salmonella* subunit antigens in chitosan-nanoparticle	Layer chickens/oral	↑ expression of toll like receptor mRNA gene expression and the proliferation of spleenocytes, but did not elucidate high titers of antibodies No significant effect on BWG or FCR	Han *et al*. (2020b)
	S. enteritidis subunit proteins, outer membrane proteins, and flagellin protein entrapped and surface flagellin protein coated chitosan nanoparticle	Layer chickens/oral	↑ mucosal IgA, but not serum IgG, IFN-γ level, lymphocyte proliferation, the toll like receptor-2 and 4, and IL-4 gene expression No significant effect on BWG or FCR	Renu *et al*. (2020a)
		Layer chickens/oral gavage or drinking water	↑ immune response ↓ cecal bacterial load No significant effect on BWG or FCR	Renu *et al*. (2020b)
	S. enteritidis encapsulated chitosan-nanoparticle vaccine	*In-ovo*	No significant effect on BWG or FCR	Acevedo-Villanueva *et al*. (2021b)
	Inactivated *S. enteritidis*, *S. typhimurium*, or *S. litchfield* heat killed antigen in chitosan nanoparticle associated with outer membrane protein and flagellin protein	Broiler chickens/oral	↑ IgY, antigen-specific protective outer membrane protein and flagellin IgA in the bile and cloaca, upregulation of toll like receptor-5 mRNA expression, and lymphocyte proliferation against *S. enteritidis* challenge ↑ antigen-specific recall response against *S. enteritidis* flagellin, *S. enteritidis*, *S. typhimurium*, and *S. litchfield* heat killed antigen ↓ cecal count of *Salmonella*	Acevedo-Villanueva *et al*. (2022)
Campylobacteriosis	Protein *Fla*A gene based chitosan-nanoparticle DNA complex vaccine	Chicken/intranasal	↑ IgG, IgA, CD4+/CD8+, and T cells ratio ↓ bacterial load	Huang *et al*. (2010)
	Recombinant hemolysin co-regulated protein of *C. jejuni* based chitosan-nanoparticle	Chicken/oral	↑ IgA, IgY, NFkB, IL-1β, IL-8, IL-6, IFN-γ, and IL-17A gene expression ↓ cecal *C. jejuni* load	Singh *et al*. (2019)
Colibacillosis	Bacteriophage encapsulated chitosan-nanoparticle	Chickens/oral	↑ body weight ↓ mortality and fecal *E. coli* shedding with a viable bacterial count in organs	Kaikabo *et al*. (2017)
Clostridiosis	Chitosan-nanoparticles loaded with native and inactivated extracellular proteins from *C. perfringens*	*In-vitro* study	Safe and immunogenic	Akerele *et al*. (2020b)
	Chitosan nanoparticle vaccine loaded with crude extracellular proteins of *C. perfringens* and *Salmonella* flagella	Broiler chickens/oral	↑ cell mediated and humoral immunity ↓ signs and mortalities Improve FCR and FI	Akerele *et al*. (2020a)
Newcastle disease	Chitosan co-administered with live NDV vaccine	Broiler chickens/oculo-nasal	↑ cellular but not humoral immunity	Rauw *et al*. (2010a)
	Herpesvirus recombinant fusion gene of NDV with chitosan	Layer chickens/oculo-nasal	↑ humoral and cellular immunity with protection against early and late infection	Rauw *et al*. (2010b)
	F gene plasmid or live NDV encapsulated in unmodified chitosan nanoparticle following oral or intranasal vaccination of chickens	Chickens/oral or Intranasal	↑ humoral systemic and local mucosal immune responses with protection against NDV challenge	Zhao *et al*. (2012 and 2014)
	O-2'-hydroxypropyl trimethyl ammonium chloride chitosan-nanoparticles live NDV vaccine	Chickens/oral or intranasal	↑ immune response	Dai *et al*. (2015)
	N-2-hydroxypropyl trimethyl ammonium chloride chitosan encapsulated attenuated live NDV	Chickens/oral or intranasal	↑ cellular and cellular immune responses ↓ NDV lesions	Zhao *et al*. (2016)
	Living NDV encapsulated	Chickens/intranasal	↑ IgG and IgA production lymphocyte	Jin *et al*. (2017)

	water soluble N-2-hydroxypropyl dimethyl ethyl ammonium chloride chitosan-nanoparticles		proliferation, and IL-2, IL-4, and IFN-γ gene expression ↓ NDV lesions	
	Modified NDV and IBV individual/or combined chitosan-nanoparticles	Chickens/intranasal	↑ IgG and IgA production, lymphocytes proliferation, and of IFN-γ, IL-2, and IL-4 gene expression ↓ NDV and IBV lesions	Zhao *et al*. (2017)
	Modified water soluble chitosan-nanoparticle encapsulated pVAX I-F(o) DNA along with C3d6 molecular adjuvant	Chickens/intranasal-intramuscular	↑ IgG, IgA, IL-2, IL-4, IFN-γ, and lymphocyte proliferation, with protection against NDV challenge	Zhao *et al*. (2018)
	O-2'-Hydroxypropyl trimethyl ammonium chloride chitosan	Chickens/intranasal	↑ IgG, IgA Lymphocyte proliferation ↑ IL-2, IL-4, IFN-γ, CD4+, and CD8 + T lymphocytes	Zhao *et al*. (2021)
Avian influenza	A mixture of inactivated AIV strains, bacterial adjuvant of *C. perfringens*, and chitosan	Layer chickens/intranasal	↑ HI antibody titers and mucosal IgA	Worrall *et al*. (2009)
	Conserved protein coated chitosan nanoparticles encapsulating AI H9N2 HA2 and M2e mRNA molecules	Layer chickens/intranasal	↑ IgG, IgA, T-cell, and cross-reactive serum virus neutralization antibody titers ↓ gross lesions in lung	Hajam *et al*. (2020)
Infectious bronchitis	An inactivated IBV encapsulated in chitosan-nanoparticles	Chickens/oculo-nasal	↓ ciliostasis, viral RNA copies number and lesions in trachea and kidney ↑ IFN-γ expression, and humoral antibodies and IgA levels	Lopes *et al*. (2018)

Conclusion

Chitosan has different promising effects on poultry production system. These effects include an improvement of production traits of broilers and layers, enhancement of antioxidant, antimicrobial, and immune responses, as well as a proper modification of various blood parameters. Besides, chitosan can be used as an excellent adjuvant for economically important poultry bacterial and viral vaccines.

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Conflict of interest

I declare that no conflict of interest could be perceived as prejudicing the impartiality of the article reported.

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