

CHITOSAN: A CRITICAL REVIEW OF CHITOSAN IMPACT IN AQUACULTURE

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*In memory of
Acad. Bogdan C. Simionescu*

Chitosan, a naturally derived cationic polysaccharide, has seen steadily increasing use across a wide range of applications, including biomedicine, environmental protection, food industry, agriculture and aquaculture. This versatility is largely attributed to its intrinsic properties, such as biocompatibility, biodegradability and bioactivity, encompassing antimicrobial, antioxidant and mucoadhesive effects. In aquaculture, chitosan has been explored both for enhancing fish growth and for improving the aquatic environment, aiming to create cleaner and healthier conditions for fish culture. Although chitosan has been extensively studied in various fields, its application in aquaculture remains relatively nascent. In this context, the present review systematically examines the literature on chitosan's impact in aquaculture, focusing on its effects on fish growth, wound healing, rescue of nervous system, antimicrobial and antiviral activities and its role in wastewater quality management.

Keywords: chitosan, aquaculture, antibacterial, antiviral

INTRODUCTION

Aquaculture, the farming of aquatic organisms for human consumption, is one of the fastest-growing food sectors, due to its relevance in food security and sustainability. Fish convert feed into protein more efficiently than terrestrial livestock, making aquaculture a more resource-efficient option in terms of land, water and feed use. This efficiency is critical in a world where population growth drives an increasing demand for animal protein. At the same time, since capture fisheries are already beyond sustainable limits, aquaculture offers a scalable source of high-quality protein, rich in omega-3 fatty acids and essential micronutrients. Moreover, aquaculture also contributes to conserving overexploited marine ecosystems, easing the pressure on declining populations.^{1,2} However, aquaculture also faces significant challenges. Excess feed, fish waste, antibiotics and antifoulants can contaminate surrounding waters, while nutrient accumulation promotes algal blooms and oxygen depletion.³⁻⁶ Dense farming conditions make farmed fish vulnerable to outbreaks of parasites and viral or bacterial diseases, while the overuse of antibiotics

contributes to the emergence of resistant pathogens, further harming ecosystems.⁷⁻¹⁰ Therefore, it is essential to develop more sustainable solutions for aquaculture protection, both fish health and environmental integrity.¹¹ The envisaged solutions include reducing antibiotic use, sustainable feed and enhancing disease prevention, among others.

Chitosan, a natural biopolymer derived mainly from the shells of crustaceans, is gaining a lot of attention in aquaculture due to its biocompatibility, biodegradability and bioactivity.¹²⁻¹⁵ In recent decades, research on chitosan in aquaculture has highlighted its multiple advantages for both fish health and water quality. Chitosan supports fish health through several pathways: it enhances immune responses, helping fish resist infections; exhibits antimicrobial activity against a broad range of bacteria, fungi and some viruses, thereby reducing disease outbreaks; and promotes tissue repair, offering protection against secondary infections.^{12,16} When used as a feed supplement, it improves gut health, nutrient absorption and feed

conversion efficiency. Moreover, unlike antibiotics, residual chitosan does not contaminate aquatic systems. On the contrary, it contributes to water purification by acting as a natural flocculant that binds suspended solids, organic matter and even heavy metals.¹⁷ However, while the chitosan advantages are in the spotlight, some limitations and disadvantages were also reported, mainly related to its solubility, variability, cost and also biological effects.^{18,19} Thus, the solubility of chitosan in acidic media makes chitosan precipitate in neutral/alkaline aquaculture waters, reducing its efficiency. The large variability of the structural parameters of chitosan (molecular weight, deacetylation degree, pattern of deacetylation, polydispersity) leads to inconsistent biologic activities and reproducibility. There are also investigations signaling a potential toxic effect of chitosan biomaterials, such as nanoparticles, on cell lines and experimental animals, such as zebrafish.¹² Furthermore, compared to conventional antibiotics, purified chitosan and related

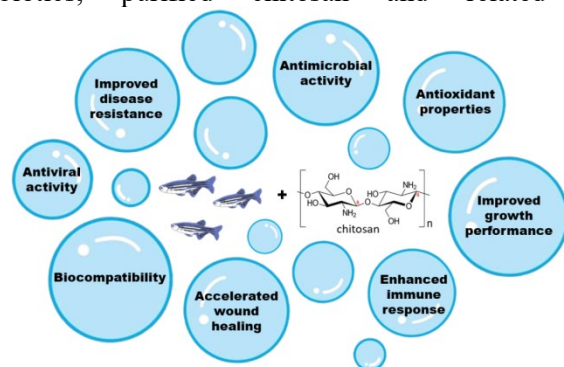


Figure 1: Advantages of chitosan-supplemented diets in aquaculture

CHITOSAN – A BIOACTIVE POLYMER IN AQUACULTURE

Chitosan is a natural biopolymer produced by the partial deacetylation of chitin, a polysaccharide found in the exoskeletons of crustaceans and insects, as well as in fungal cell walls.²⁰ Its structure consists mainly of β -(1 $\rightarrow$ 4)-linked D-glucosamine units, with varying proportions of N-acetyl-D-glucosamine, the degree of deacetylation (DD) typically ranging from 50% to 95%. This parameter strongly influences chitosan's solubility and biological activity. Owing to its high content of glucosamine units, chitosan is the only naturally occurring cationic polysaccharide, although this

formulations can be expensive, limiting its large scale application.

In this context, the present review focuses on the impact of chitosan and chitosan-based formulations in aquaculture. Figure 1 provides a synthesized overview of chitosan's effects on fish, serving as a visual summary of its biological impact. These aspects will be further elaborated in the subsequent sections.

Methodology

The articles included in this review were selected from the ISI Web of Science database using the keywords "aquaculture" and "chitosan" (753 results; Fig. 2). The results were filtered by relevance and articles were screened to include only those that mentioned both keywords in the abstract. Selected articles were then systematically reviewed and the most relevant findings were organized into subchapters to facilitate readability and comprehension.

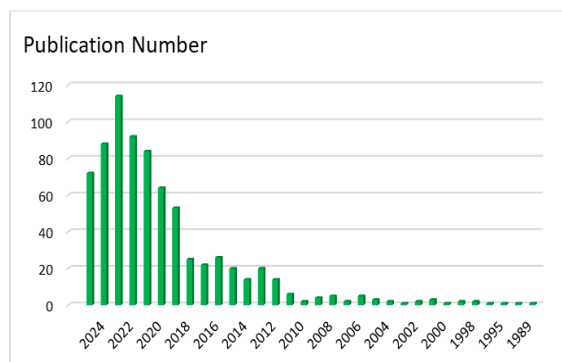


Figure 2: Distribution of articles published during 1989–2024, found in ISI Web of Science using the keywords "chitosan" + "aquaculture"

polycationic character manifests only in aqueous acidic media, where amino groups become protonated. Protonation disrupts interchain hydrogen bonding and increases chain mobility, resulting in improved solubility, swelling and pH-responsiveness. In alkaline conditions, by contrast, amino groups are deprotonated, promoting interchain hydrogen bonding, enhanced structural integrity and reduced solubility.

Chitosan is generally regarded as a non-toxic biopolymer due to its natural origin, but its safety profile depends on factors such as molecular weight, DD, concentration, dosage, formulation and administration route. For example, intranasal

or intravenous delivery can elicit immune responses or mucosal irritation, effects not observed with topical applications.²¹ Higher DD chitosan tends to be more cytotoxic because of its increased cationic charge density, which may disrupt cell membranes.

Chitosan is biodegradable, predominantly via enzymatic hydrolysis by endogenous enzymes present in biological fluids and biocompatible, owing to its structural similarity to glycosaminoglycans and favorable interactions with biological systems. It is also non-allergenic and its degradation products have been reported to support tissue regeneration and immunomodulation. These attributes are advantageous in applications such as aquaculture, where chitosan does not accumulate in fish and may even contribute to tissue health. However, high-DD chitosan at elevated concentrations can cause hemolysis or cytotoxicity, although such exposures are unlikely under typical aquaculture conditions.²²

Chitosan is recognized as an antimicrobial agent, having the ability to contribute to disease prevention in fish. However, its therapeutic activity is limited and can be overcome in formulations with drugs or other bioactive agents to achieve antimicrobial compounds for aquatic environments. The cationic character and the multitude of hydroxyl units endow chitosan with mucoadhesivity, a property which can facilitate its uptake across fish mucosal surfaces, improving bioavailability and treatment efficacy, thus enabling lower dosing.²³

Chitosan exhibits notable antioxidant activity, which is primarily attributed to the free amino and hydroxyl groups along its backbone.^{24,25} These functional groups can scavenge reactive oxygen species (ROS), chelate transition metals that catalyze radical formation and inhibit lipid peroxidation. This can help reduce oxidative stress in fish, which is often elevated under intensive rearing conditions, pathogen exposure, or environmental fluctuations.²⁶ Lower oxidative stress translates into improved immune function, higher survival rates and enhanced growth performance. The antioxidant character of chitosan is also helpful in formulation of feed as protectant for sensitive bioactive compounds (such as vitamins, probiotics, or vaccines) from oxidative degradation, thereby maintaining their efficacy during storage and delivery.²⁷ Moreover, the incorporation of chitosan in coatings, nanoparticles, or hydrogels can provide a dual

benefit, antimicrobial protection and antioxidant support, aligning with the goals of sustainable aquaculture by reducing the need for synthetic additives and improving overall fish health.^{28,29}

Chitosan has gained significant attention as a natural immunoadjuvant, attributed to its cationic nature, which favors the interaction with negatively charged mucosal surfaces and cell membranes, facilitating antigen uptake and immune activation. Chitosan has been shown to stimulate the release of cytokines and activate pathways involved in both humoral and cell-mediated immunity.²⁵ This property is relevant in aquaculture, where fish are constantly exposed to environmental stressors and a wide range of pathogens.¹⁵ Chitosan's immunoadjuvant activity offers a promising approach to strengthen fish immunity in a safe and sustainable manner, helping for maintaining health and reducing reliance on antibiotics.³⁰

An important advantage of chitosan is its ability to be manufactured as biomaterials, such as nanoparticles, hydrogels, films and nanofibers, which can be optimized as delivery platforms.³¹ Embedding natural bioactive compounds into chitosan biomaterials, including essential oils and plant extracts, is an emerging trend for developing eco-friendly, synergistic formulations that support fish health and performance, while aligning with sustainable aquaculture practices.

CHITOSAN AS MULTIFUNCTIONAL FEED IN AQUACULTURE

Research on chitosan in aquaculture has highlighted its dual role as a nutritional enhancer and a functional additive that promotes fish health, growth and resilience.³² Its polycationic nature and bioactive properties enable modulation of physiological processes that directly or indirectly influence fish growth. Chitosan interacts with the mucosal surfaces of the gastrointestinal tract, increasing mucosal permeability and enhancing nutrient absorption. This mucoadhesive property, combined with its ability to form stable complexes with nutrients or bioactive compounds, can improve feed efficiency and weight gain.^{17,32,33} Moreover, chitosan exhibits broad-spectrum antimicrobial activity, which supports beneficial gut microflora and overall gut health. A healthier intestinal environment enhances digestion and feed conversion, critical factors for growth performance. In line with this, chitosan-based nanoparticles and coatings are increasingly

employed as delivery systems for probiotics, vaccines and micronutrients in aquaculture.³³ Additionally, chitosan's intrinsic antioxidant, immunostimulant and antimicrobial properties further optimize health and growth outcomes, while reducing stress and reliance on pharmaceuticals.

Multiple studies have demonstrated that chitosan administration enhances the mucoadhesive potential of feed, improving intestinal absorption and oral bioavailability. As a result, chitosan-supplemented diets have been shown to boost growth, survival and meat quality across various freshwater and marine species, including rainbow trout, olive flounder, koi, kelp grouper, turbot, gibel carp, mrigal carp, Asian seabass and sea bass.³⁴ Furthermore, chitosan nanoparticles have proven more effective than conventional chitosan in enhancing growth performance and food quality in Nile tilapia and African catfish fingerlings.^{15,34} Chitosan coated alginate beads encapsulating probiotics showed improved stability in seawater and controlled release of viable probiotics for growth of black-footed abalone (*Haliotis iris*) (Fig. 3).³⁵

Other investigations demonstrated that oral administration of chitosan to Atlantic salmon

modulated gut microbiota and enzyme production,³⁶ and modified the intestinal morphology,³⁷ consequently favoring fish growth.

In a study, Chen *et al.* investigated the effects of dietary chitosan supplementation on juvenile *Carassius auratus*. Diets were fortified with graded levels of chitosan (1,800, 4,000, 7,500, 10,000 and 20,000 mg/kg of diet) and administered over a 75-day period. The findings indicated that 4,000 mg/kg diet represented the optimal supplementation level, yielding the highest growth performance. This enhancement was associated with improvements in intestinal morphology, specifically the proliferation of microvilli and the abundance of goblet cells within the epithelial lining³⁸ (Figs. 4, 5).

Furthermore, the encapsulation of essential oils into chitosan nanoparticles has been shown to enhance humoral immune responses and growth performance in different fish species, specifically *Mentha piperita* when investigated in Siberian sturgeon (*Acipenser baeri*),³⁹ lemon when investigated in rainbow trout,⁴⁰ *Euterpe oleracea* when investigated on tambaqui (*Colossoma macropomum*) production⁴¹ and thymol when investigated in Nile tilapia.⁴²

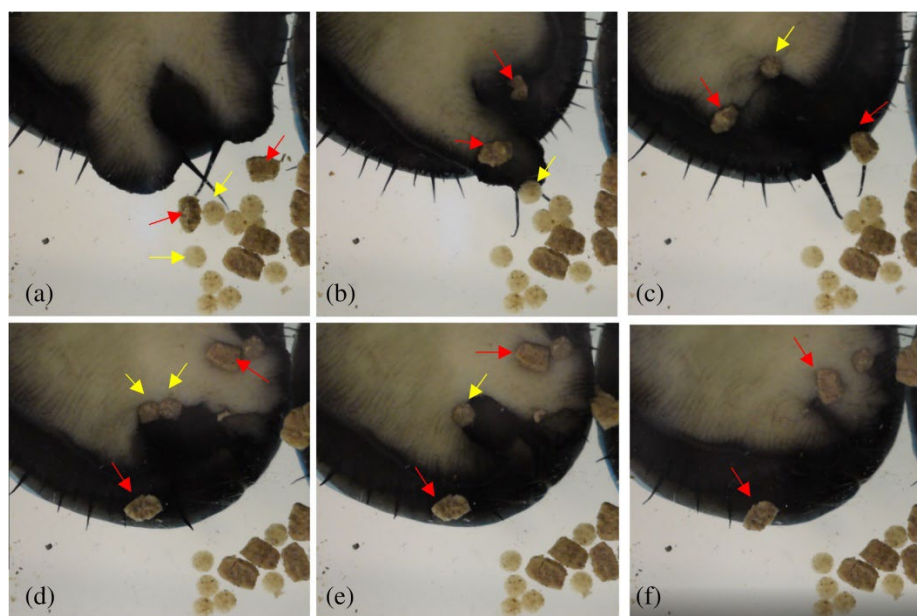


Figure 3: Feeding behavior of abalone when offered two different feed types: (a) initial food sensing, with probiotics-encapsulated chitosan–alginate beads (yellow arrows) and commercial feed (red arrows) detected; (b) grasping two commercial feed pellets and one chitosan–alginate bead using the foot muscle; (c) no consumption observed at this stage; (d) increased capture of chitosan–alginate beads; (e) active consumption of beads; (f) several chitosan–alginate beads consumed, while the commercial feed remained uneaten³⁵

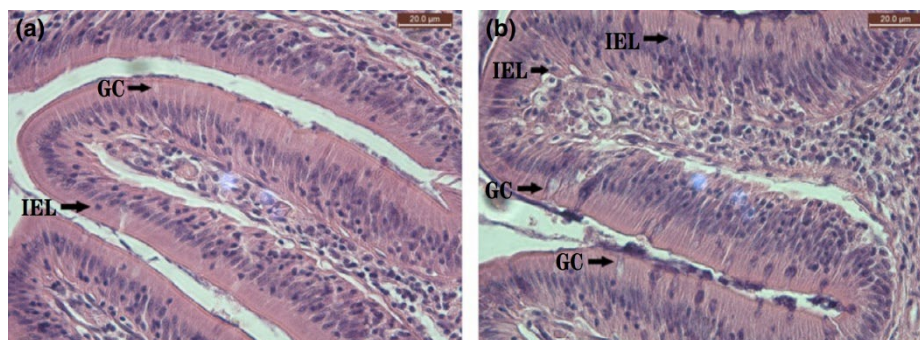


Figure 4: Histological photomicrographs of intestinal epithelium in juvenile *Carassius auratus*: (a) control group displaying normal microvilli structure with few goblet cells; (b) group supplemented with 4,000 mg chitosan/kg diet showing increased microvilli length, a higher density of goblet cells and the presence of intraepithelial lymphocytes³⁸

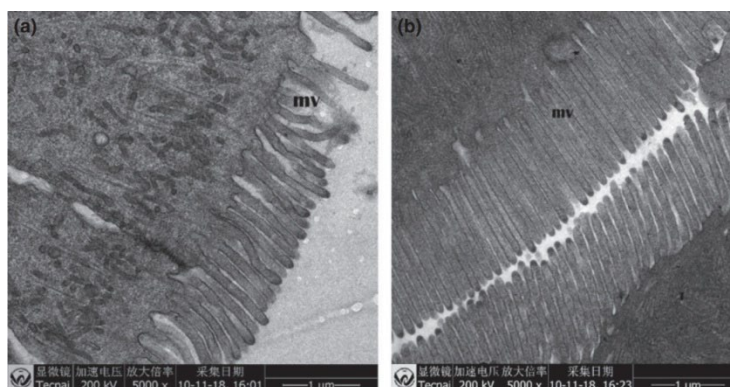


Figure 5: Transmission electron micrographs of intestinal microvilli in juvenile *Carassius auratus*: (a) control group showing microvilli of normal length; (b) group supplemented with 4,000 mg chitosan/kg diet exhibiting pronounced proliferation of microvilli³⁸

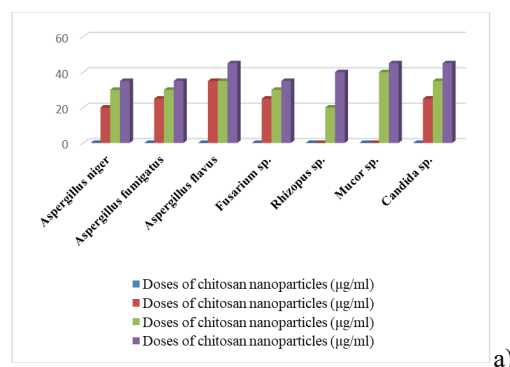
CHITOSAN AS ANTIMICROBIAL AGENT IN AQUACULTURE

Considering the rising concerns over antibiotic resistance and environmental sustainability, chitosan has emerged as a promising natural alternative for disease control in aquaculture, due to its inherent antimicrobial activity. Chitosan exhibits strong activity against a wide range of aquatic pathogens, including Gram-positive and Gram-negative bacteria, fungi and certain viruses. Its mechanism involves disrupting microbial cell membranes, binding to negatively charged surfaces and interfering with nutrient uptake and gene expression.⁴³ This multi-targeted action reduces the risk of resistance development and enhances overall pathogen control. This was confirmed by investigations on specific fish species. Thus, administered in warmwater fishes, chitosan exhibited *in vitro* dose-dependent antibacterial activity against *A. hydrophila*, *Edwardsiella ictaluri* and *Flavobacterium columnare*.⁴⁴ Moreover, chitosan oligomers showed better activity, mainly correlated with their higher solubility, which improved the bioavailability and exhibited a higher ability to

interact with bacterial surfaces *via* adsorption. Formulated as nanoparticles, chitosan exhibited antibacterial activity towards different geographical isolates of *Aeromonas hydrophila*, improving health status and disease resistance in tropical rohu fish.⁴⁵

A comprehensive *in vitro* investigation conducted to evaluate the antimicrobial efficacy of chitosan nanoparticles against a range of fungal and bacterial strains isolated from both diseased and healthy specimens of Nile tilapia (*O. niloticus*) brought interesting results.⁴⁶ It was revealed that chitosan nanoparticles inhibited the growth of all tested strains in a dose-dependent manner. Notably, fungal strains exhibited greater resistance, requiring four times higher concentrations compared to bacterial strains. Among the fungal isolates, *A. flavus*, *Mucor* spp. and *Candida* spp. were more susceptible to chitosan treatment compared to *A. niger*, *A. fumigatus* and *Fusarium* spp., which showed higher resistance (Fig. 6a). In the case of bacterial isolates, *A. sobria*, *A. hydrophila* and *P. aeruginosa* displayed the largest inhibition zones,

while *S. aureus* and *P. fluorescens* were



comparatively less sensitive (Fig. 6b).

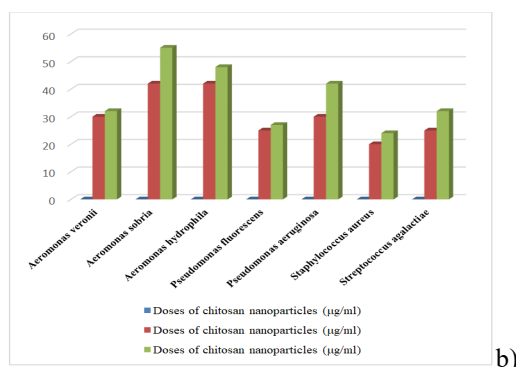


Figure 6: Inhibition zones (mm) of *in vitro* (a) fungal and (b) bacterial growth at varying concentrations of chitosan nanoparticles ($\mu\text{g/mL}$), following 48 h of incubation at 30 °C (adapted from⁴⁶)

Other interesting investigations showed that the antibacterial activity of chitosan can be further improved by combination with various bioactive agents, such as metal nanoparticles, phytochemicals and essential oils. In this line of thought, chitosan-silver nanoparticles, proved *in vitro* activity against several fish-sepsis-causing bacteria, such as *A. hydrophila*, *E. tarda*, *P. piscicida*, *P. aeruginosa*, *S. faecium*, *S. iniae*, *V. ordalli*, *V. anguillarum* and *Y. Ruckeri*.⁴⁷ Furthermore, a more potent antimicrobial effect was recorded by combining these nanoparticles with conventional antibiotics in aquaculture, such as amikacin, kanamycin, florfenicol, erythromycin, ciprofloxacin, rifampicin, tetracycline, oxytetracycline and minocycline,⁴⁷ reaching a synergistic effect in the case of amikacin and rifampicin.^{47,48} In other studies, ZnO nanoparticles or AgCl co-doped ZnO nanoparticles were encapsulated into chitosan hydrogels, which showed *in vitro* antimicrobial activity against *V. harveyi*.⁴⁹

Further improvements in antibacterial efficacy were achieved by conjugating chitosan with phytochemicals, such as ferulic acid, sinapic acid and caffeic acid. These complexes exhibited strong activity against antibiotic-resistant fish pathogens, including *E. tarda*, *V. harveyi* and *P. damsela*, isolated from Korean cultured fish.⁵⁰ Additionally, these formulations demonstrated synergistic interactions with antibiotics like erythromycin and oxytetracycline, lowering MIC values and enhancing bacterial susceptibility.

An ecofriendly approach consisted in developing eco-friendly and effective antimicrobial agents by encapsulating essential

oils (EOs) with known antimicrobial properties, into chitosan nanoparticles.^{51–53} This strategy has the potential not only to improve the physicochemical stability and bioavailability of EOs, but also to enhance their therapeutic potential through synergistic effects. In this context, numerous EO-based chitosan formulations have been evaluated across various fish species. For example, dietary supplementation with pumpkin seed oil-loaded chitosan nanoparticles significantly improved growth performance and immune response in *L. vannamei* shrimp, while conferring protection against *V. parahaemolyticus*, reducing pathological damage to muscle, intestinal and hepatopancreas tissues compared to chitosan alone.⁵⁴

The hepatopancreas plays a critical role in nutrient absorption, storage and the synthesis of digestive enzymes. The effects of dietary chitosan nanoparticles (ChNPs/PSO) supplementation on the hepatopancreatic tissues of *L. vannamei* following *Vibrio parahaemolyticus* challenge are shown in Figure 7 (A–D). In the ChNPs+PSO2 group (Fig. 7D), marked improvements were observed in the hepatopancreatic tubules, as well as in B-cells and F-cells. The ChNPs+PSO1.5 group (Fig. 7C) exhibited mild destruction of the tubules with moderate recovery of B- and F-cells. Moderate tubular damage and erosion of some hepatopancreatic tubules were evident in the ChNPs+PSO1 group (Fig. 7B). In contrast, the infected control group (ChNPs only; Fig. 7A) displayed pronounced deformities and severe erosion of the hepatopancreatic tubules, indicating infection-induced tissue deterioration.⁵⁴

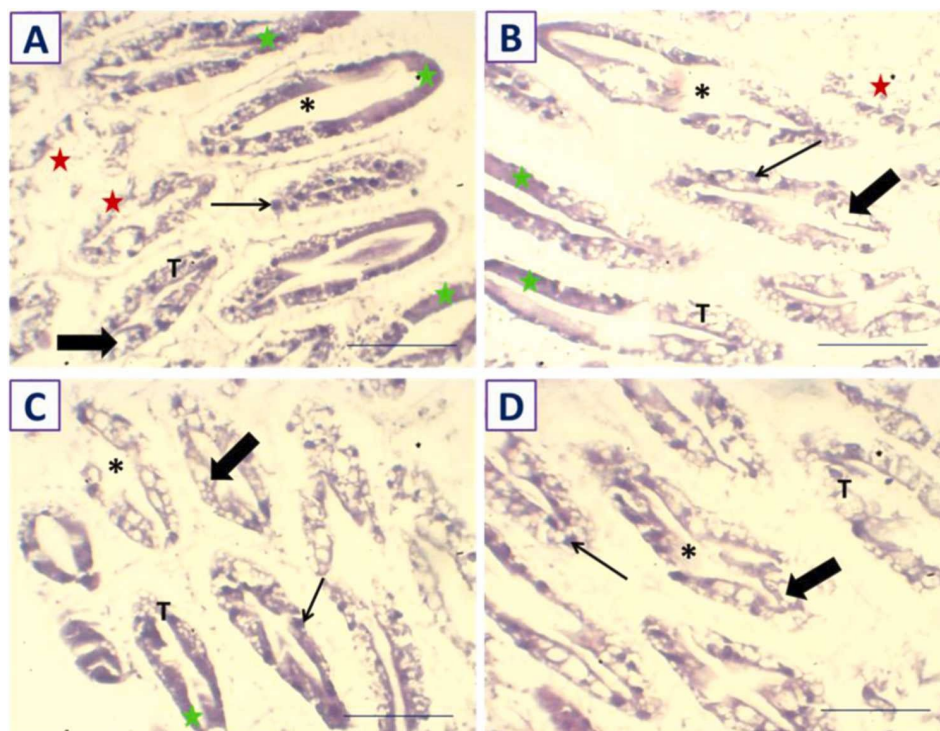


Figure 7: Effects of dietary chitosan nanoparticles (ChNPs) and pumpkin essential oil-loaded chitosan nanoparticles (PSO/ChNPs) on the hepatopancreatic tissues of *L. vannamei* following infection; (A) Control group fed the basal diet with 1 g ChNPs; (B–D) Shrimp fed the control diet supplemented with PSO/ChNPs at 1 (ChNPs+PSO1; B), 1.5 (ChNPs+PSO1.5; C) and 2 (ChNPs+PSO2; D) mL kg⁻¹ diet, respectively (histological features include B-cells (thin arrows), F-cells (thick arrows), hepatopancreatic tubules (T), lumen (asterisks), degeneration of hepatopancreatic tubule cells (green stars) and erosion of hepatopancreatic tubules (red stars); [H&E staining; scale bar = 100 μm]⁵⁴)

USE OF CHITOSAN IN VACCINATION

Oral administration is considered the most practical and efficient strategy for mass vaccination in aquaculture, providing a stress-free and scalable means of immunizing fish populations. Nonetheless, plasmid-based vaccines encounter significant limitations when delivered orally, as they are rapidly degraded in the harsh conditions of the gastrointestinal tract. Encapsulation within nanoparticles offers a promising approach to address this challenge. By shielding the genetic material, nanoparticle carriers protect the vaccine from enzymatic degradation and ensure its stability during feed processing.

Selecting suitable carriers for vaccines in aquaculture remains a considerable challenge, as they must ensure both safety and biocompatibility in fish.⁵⁵ Chitosan has shown strong potential not only as a delivery vehicle, but also as a functional adjuvant, capable of enhancing both mucosal and systemic immune responses and thereby improving survival rates against bacterial and viral infections. Several studies have demonstrated that chitosan-based nanovaccines

can be safely administered via immersion, resulting in improved immunological parameters, including increased antibody production and enhanced antioxidant enzyme activity.

With regard to the intrinsic antiviral properties of chitosan in aquatic species, dietary administration of chitin has been shown to stimulate the immune response of *Macrobrachium rosenbergii* and confer resistance against extra small virus and nodavirus.⁵⁶ Similarly, injectable administration of chitin and chitosan in *Litopenaeus vannamei* (white shrimp) enhanced immune defenses and increased resistance to *Vibrio alginolyticus* infection. Immunological assessments revealed elevated total hemocyte counts, increased phenoloxidase activity, enhanced respiratory burst and greater phagocytic activity. Notably, chitosan demonstrated greater efficiency than chitin, achieving comparable immunostimulatory effects at lower doses.⁵⁷

The significance of chitosan in antiviral therapy lies largely in its ability to form complexes with a wide range of bioactive compounds and its mucoadhesive properties,

which prolong residence time at mucosal surfaces and thereby enhance delivery efficiency. These characteristics render chitosan an effective carrier for antiviral agents, a role supported by numerous studies that highlight its versatile applications in this context.

A study on the oral delivery of a DNA vaccine encapsulated in chitosan-TPP nanoparticles for protection against nodavirus in Asian sea bass (*Lates calcarifer*) reported a 60% survival rate following viral challenge via intramuscular injection in vaccinated fish.⁵² Biodistribution analysis confirmed successful gene delivery to multiple tissues, including the heart, intestine, gills and muscle. The nanoparticles, characterized by high encapsulation efficiency, exhibited low cytotoxicity, maintaining over 90% cell viability in sea bass kidney cells, as demonstrated by the MTT assay.

Another study evaluated the efficacy of chitosan nanoparticles encapsulating recombinant outer membrane protein A of *Edwardsiella tarda* as an oral vaccine in fringed-lipped peninsula carp (*Labeo fimbriatus*).⁵⁸ In comparison with an inactivated whole-cell vaccine, chitosan nanoparticles induced higher antibody responses and conferred greater protection against *E. tarda*-induced mortality, underscoring the advantages of chitosan-based encapsulation.

A plasmid DNA encoding the major capsid protein (MCP) gene of lymphocystis disease virus was encapsulated in chitosan microspheres using an emulsion-based technique and evaluated as an oral vaccine in Japanese flounder (*Paralichthys olivaceus*).⁵⁹ Reverse transcription PCR analysis demonstrated that chitosan encapsulation prolonged the presence of MCP gene transcripts in gill, intestinal, renal and splenic tissues, extending from 10 to 90 days post-vaccination. Moreover, fish immunized with the chitosan-encapsulated plasmid exhibited a sustained humoral immune response, with detectable serum antibody levels from week 1 through week 16, significantly surpassing the duration observed in fish vaccinated with naked plasmid DNA.

Biodegradable microspheres composed of chitosan and Gantrez, covalently conjugated with surface antigens of *Philasterides dicentrarchi*, were evaluated for their efficacy in mitigating the effects of this histiophagous scuticociliate endoparasite in turbot aquaculture.⁶⁰ A comprehensive comparative study highlighted the strong adjuvant potential of these microspheres in enhancing the innate immune response,

supporting their use in the development of safe and effective vaccines against fish pathogens.

An oral DNA vaccine formulated with chitosan nanoparticles against nodavirus in European sea bass markedly upregulated genes associated with cell-mediated cytotoxicity and the interferon response in the gut, resulting in improved survival rates following nodavirus infection.⁶¹ Although this oral vaccine does not induce specific antibodies, the upregulation of immune-related genes in the gut confers partial protection and enhances disease resistance.

Notably, an investigation demonstrated the potential of orally delivered DNA adjuvants to improve vaccine performance in fish, providing a practical alternative to injection-based methods.⁶² In this study, chitosan nanoparticles encapsulating a DNA adjuvant encoding alphavirus replicase (NP-Ad) were co-administered with an inactivated ISAV antigen (NP-V) to Atlantic salmon. While NP-V alone provided limited protection, co-administration with NP-Ad significantly enhanced efficacy, achieving a 77% protection rate.

Comparative studies on microsphere-based vaccine carriers have highlighted the superior performance of chitosan relative to other polymers. In particular, microspheres composed of a ternary blend of alginate, chitosan and PLGA, encapsulating the outer membrane protein antigen of *Aeromonas hydrophila*, demonstrated enhanced encapsulation efficiency and elicited stronger antibody responses compared with PLGA-only formulations in a fish model.⁶³ The composite microspheres induced a significantly elevated specific antibody response that persisted for up to nine weeks post-immunization. This prolonged immune activation, involving both innate and adaptive pathways, conferred effective protection against *A. hydrophila* infection following intraperitoneal administration.

A key challenge in oral vaccination is the development of formulations that are convenient to administer. Studies have demonstrated that chitosan nanoparticles can be effectively incorporated into feed pellets, enabling efficient oral delivery of plasmid DNA (pDNA). This strategy is particularly valuable for on-farm mass immunization, especially for DNA-based vaccines and small fish species, for which intramuscular administration is impractical.^{64,65}

CHITOSAN IN WOUND HEALING AND TISSUE REGENERATION

Chitosan exhibits remarkable wound healing properties, as demonstrated in numerous studies involving both the native polymer and its derivatives or biomaterial composites.^{66,67} This characteristic holds particular relevance in aquaculture, where the presence of chitosan in water may aid in the recovery of injuries that naturally occur among fish. By promoting tissue repair, chitosan can potentially extend fish lifespan and mitigate secondary effects associated with fish mortality, such as algal blooms or bacterial contamination.⁶⁸

Experimental studies using zebrafish models have shown that chitosan significantly enhances

wound healing by accelerating wound closure, stimulating tissue regeneration and exerting anti-inflammatory effects. These outcomes were supported by the modulation of key inflammatory cytokines, including Bax, Bcl2, IL-2, IL-6 and TNF- α ⁶⁹ (Fig. 8). However, it is important to note that the concentration of chitosan used in these studies was relatively high, around 30 mg/L, which may pose cost-related challenges for large-scale aquaculture applications. Interestingly, the healing response was even more pronounced when using zinc-loaded chitosan nanoparticles, suggesting a promising avenue for enhanced therapeutic efficacy.

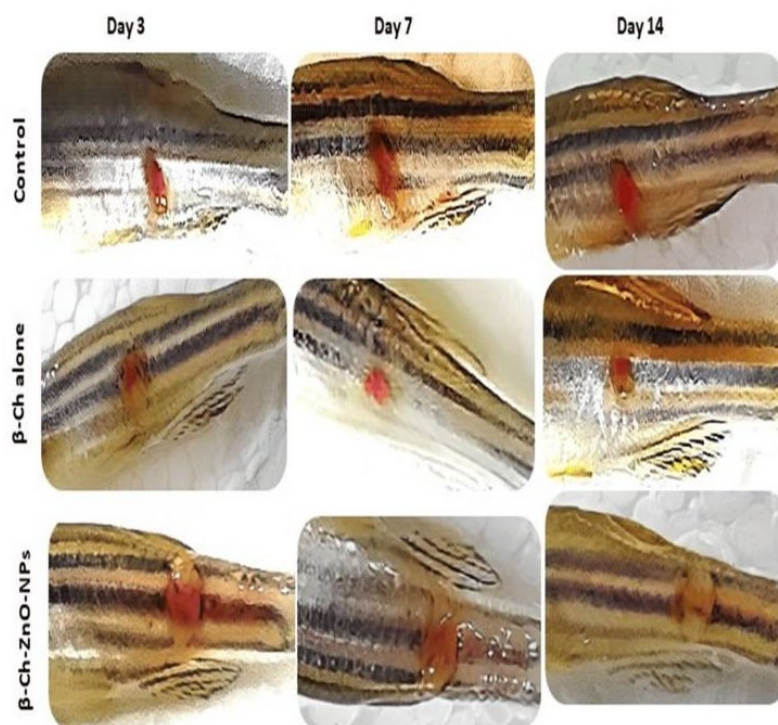


Figure 8: Images collected over 15 days after inflicting wounds on zebrafish and administration of chitosan and chitosan-derived zinc oxide nanoparticles⁶⁹

Other metal–chitosan nanoparticles, such as copper–chitosan (Cu–chitosan), have exhibited wound healing effects comparable to those of zinc–chitosan (Zn–chitosan) nanoparticles, even at lower concentrations of 20 mg L⁻¹ and 10 mg L⁻¹, respectively. These effects are primarily attributed to their ability to modulate inflammation and oxidative stress, both of which play central roles in the wound healing process.⁷⁰

Chitosan-based hydrogels incorporating Asiatic acid, a potent antioxidant with antimicrobial activity and the ability to disrupt

mature biofilms, have shown promising wound healing effects, while remaining cytocompatible with fibroblasts. The inclusion of Asiatic acid in the hydrogel formulation significantly enhanced chitosan's wound healing performance by accelerating wound closure and promoting tissue remodeling in zebrafish previously infected with MRSA.⁷¹ Despite the relevance of this approach for wound dressing development, direct application of hydrogels to fish wounds may be impractical in aquaculture, where systemic or waterborne delivery methods are more feasible.

Nevertheless, the improved mucoadhesivity of chitosan hydrogels allows them to adhere effectively to the mucus-rich skin of fish, enhancing the delivery of bioactive agents.⁷² In addition, their capacity to act as flocculants, binding suspended solids, heavy metals and organic pollutants, further underscores their multifunctional potential in aquaculture applications.^{72,73} Chitosan nanofibers can be also an alternative for wound healing, as they show high surface to volume ratio and consequently high active surface, even though routes for their administration in fish can be challenging.⁷⁴

NERVOUS SYSTEM RECOVERY

The nervous system of fish is highly susceptible to a variety of environmental stressors and pollutants. Neurological damage can result from the bioaccumulation of heavy metals, such

as mercury, lead and cadmium, as well as exposure to pesticides, industrial chemicals (e.g., organophosphates and PCBs) and pharmaceutical residues in wastewater, including antidepressants and antibiotics.⁷⁵

Chitosan has shown promise in mitigating neurodegenerative effects by reducing oxidative stress, modulating inflammatory pathways and promoting tissue regeneration.⁷⁶ In zebrafish embryo models of neural injury, chitosan-based hydrogels were reported to partially restore the central nervous system function.⁷⁷ Further investigations using chitosan–hyaluronan hydrogels demonstrated enhanced spreading, migration, proliferation and differentiation of encapsulated neural stem cells *in vitro*, along with significant injury repair and functional recovery in adult zebrafish models of traumatic brain injury (Fig. 9).⁷⁸

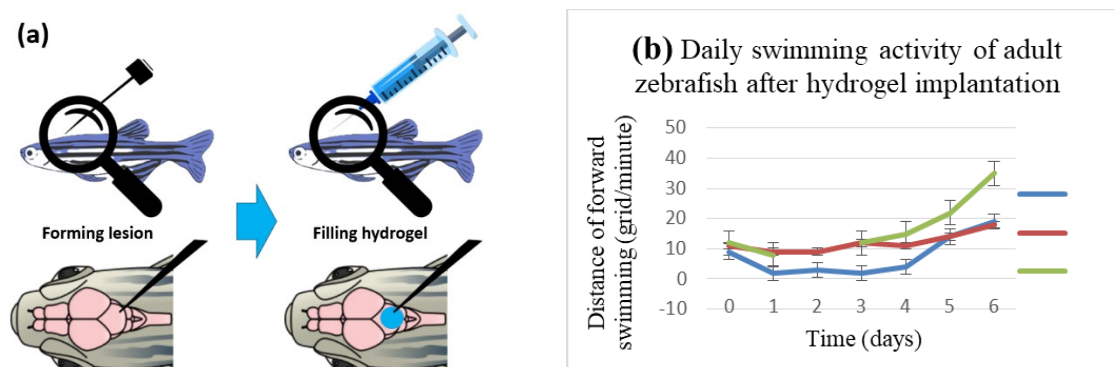


Figure 9: Functional recovery following implantation of CS or CH hydrogel in a zebrafish TBI model; (a) Schematic of the TBI model – a cranial lesion was created in the central encephalon (CE) using a 26-gauge syringe needle, followed by hydrogel injection into the lesion; (b) Daily swimming activity of adult zebrafish after hydrogel implantation (the control group received PBS (pH 7.4); CS: chitosan hydrogel; CSH: chitosan–hyaluronan interpenetrating network; adapted from ⁷⁸)

Conversely, chitosan nanoparticles have exhibited dose-dependent toxicity in zebrafish embryos, resulting in notable neurobehavioral alterations. Treated embryos displayed reduced spontaneous movement, while larvae showed signs of hyperactivity. Additional adverse effects included decreased hatching rates, elevated mortality and a higher incidence of developmental malformations, such as impaired axonal development of primary and secondary motor neurons and altered muscle structure.⁷⁹ These effects are attributed to the small size and large surface area of the nanoparticles, which facilitate their passage across the blood–brain barrier, further enhanced by the polycationic nature of chitosan. While this property could be

advantageous for developing drug delivery carriers for neurological disorders, it also poses a risk of neurotoxicity. Notably, such effects have primarily been observed in zebrafish embryo models, which are highly sensitive. Interestingly, the literature is somewhat contradictory, as other studies have reported that incorporating the neurotoxic drug carbamazepine into chitosan nanoparticles significantly reduced its toxicity and adverse neurological effects in seizure models.⁸⁰

CHITOSAN IN WASTEWATER MANAGEMENT

The removal of organic compounds, inorganic nutrients and bacteria from aquaculture

wastewater prior to discharge not only minimizes the deterioration of receiving water quality, but also enables the potential reuse of the water. This is particularly important because aquaculture systems generate ammonia nitrogen as a byproduct of protein metabolism in aquatic animals.¹² If such effluents are not properly managed, eutrophication can occur, negatively affecting the survival and growth rates of cultured species.

Chitosan has been recognized as an effective agent for improving aquaculture wastewater quality, demonstrating strong potential in the removal of suspended solids, organic compounds, ammonia (NH_3), phosphate (PO_4^{3-}) and pathogens such as *Edwardsiella ictaluri*, while leaving beneficial nitrifying bacteria largely unaffected.⁸² Treatment efficiency was shown to depend on the structural parameters of chitosan and the pH of the wastewater, with higher deacetylation degree (DD), higher molecular weight (Mw) and lower pH enhancing performance due to the increased

polycationic character of chitosan under these conditions.^{82,83}

Composite beads of chitosan and bentonite clay have been investigated, exhibiting high efficiency in ammonia nitrogen removal.⁸⁴ Further improvements were achieved by transforming chitosan into a foam incorporated into filters, which could subsequently be used as fertilizers once no longer suitable as a filtration medium, thereby closing the sustainability loop in aquaculture production.⁸⁵ Building on these approaches, recovery of floc from shrimp aquaculture wastewater using chitosan was applied as an organic fertilizer for *Ipomoea aquatica* (water spinach). This study demonstrated that chitosan-derived floc outperformed other treatments, resulting in greater root volume and length, plant height, leaf count and fresh and dry biomass, indicating that nutrients were readily available for plant absorption and growth.⁸⁶



Figure 10: Photo of terrestrial aquaculture plants⁸¹



Figure 11: Matured *Ipomoea aquatica* at harvest (55 days) from five treatments (T1 sand; T2: sand + fertilizer; T3: sand +floc; T4: garden soil)⁸⁶

The concept of a sustainable cycle has also been explored in the context of recirculating aquaculture systems, employing approaches that enable efficient wastewater treatment for reuse, while simultaneously producing biomass that can be harvested as a protein-rich, immune-stimulating fish feed without the use of antibiotics. In this context, the addition of small amounts of medium-molecular-weight chitosan significantly enhanced the harvest efficiency of polyhydroxybutyrate (PHB)-rich *Zoogloea denitrificans* within these systems.⁸⁷

An innovative approach for managing excessive nutrient concentrations in mariculture effluent involves bioremediation through the combined use of degrading bacteria and macroalgae. In this system, the bacteria convert ammonia and nitrite into nitrate and reduce chemical oxygen demand, while the macroalgae utilize nitrate and phosphate as nutrients for growth. Together, these organisms act synergistically to lower nitrogen and phosphorus concentrations in the effluent.⁸⁸

As aquaculture organisms grow, their sensitivity to ammonia nitrogen and nitrite increases, disrupting enzymatic hydrolysis and membrane stability, leading to gill rot, weakened immunity, stunted growth and potentially death.⁸⁹ Comparative experiments, *in vitro* and in real aquaculture samples, carried out with different adsorbent systems containing chitosan, gelatin and alginate to evaluate their potential in removal toxic heavy metals, such as Pb, Cu, Cd, Zn, Ni, As, Hg, proved chitosan as the most recommended for multiple cycles of water remediation treatments.⁷²

Chitosan also proved efficiency as a natural biocoagulant to harvest *Chlorella* sp., a dominant microalgae species in bioflocs, which maintain the quality of water in aquaculture systems at a safe level, by reducing nutrient pollutant.⁹⁰ Good results were also obtained on unicellular *Cyanobacterium Synechocystis* sp. used for phosphate removal in a recirculating aquaculture system, when chitosan solutions proved good cell harvesting ability.⁹¹ In view of recirculating the aquaculture system, chitosan has been used in order to flocculate phytoplankton, considering that the removal phytoplankton cells leads to the reduction of nitrogenous waste and improves water quality.⁹²

Antibiotics are used to reduce bacterial infections in aquaculture. However, due to the

potential negative effects of antibiotics, such as the development of resistant strains, environmental contamination and posing a threat to food safety and reduction in intestinal microbial diversity of fishes, this relative practice is recommended to be avoided and has become increasingly controversial around the world. Chitosan and chitosan oligomers showed antimicrobial properties toward known aquaculture pathogens (*Vibrio campbellii*, *Aeromonas hydrophila* and *Streptococcus agalactiae*) and non-pathogens (non-pathogenic *Escherichia coli*, *Bacillus megaterium* and *Rhodococcus jostii* RHA1), at low doses, constituting an alternative to antibiotics, with potential of flocculant as well.⁹³

Nanoparticles of chitosan-selenium nanoflower showed dose dependent toxicity against isolated fish pathogen *Aeromonas caviae*, one of the most common aquatic pathogens that infect the majority of economically important fishes, causing significant losses to the aquaculture industry. It showed maximum cytotoxicity of 75.06% at 1000 μ g/mL of *Aeromonas caviae* bacteria.

Chitosan nanofibers embedding silver nanoparticles also exhibited activity against a broad spectrum of bacteria commonly found in fish. They successfully inhibited the growth of fish pathogenic bacteria *Vibrio* (*Allivibrio*) *salmonicida*, *V. tapetis*, *Edwardsiella tarda* and fungi *Fusarium oxysporum*, and significantly diminished *V. salmonicida* concentration in artificial seawater, being proposed as functional antimicrobial filters.⁹⁴ Overall, chitosan nanofibers reinforced with metal nanoparticles, such as copper, copper oxide, zinc or zinc oxide, appear as a promising approach for bioactive filters in aquaculture.⁹⁵⁻⁹⁷

CONCLUSION

Chitosan is a versatile biopolymer with significant potential for multi-level applications in aquaculture water treatment. It effectively removes pollutants through flocculation, enhancing water quality and reducing environmental stress. Additionally, chitosan supports fish health by boosting immune responses and lowering oxidative stress, thereby minimizing susceptibility to opportunistic pathogens and promoting improved growth performance.

The integration of chitosan into aquaculture systems represents a compelling example of how bio-based innovations can drive sustainability within the framework of the circular bioeconomy. Derived from crustacean waste, an abundant and underutilized byproduct, chitosan transforms marine biomass into a high-value functional material, aligning with the principles of resource efficiency, waste valorization and ecological stewardship.

Beyond its origin, chitosan exhibits a multifunctional profile that supports both fish health and environmental quality. Its antimicrobial, immunostimulatory, antioxidant and growth-promoting properties contribute to enhanced disease resistance, improved physiological performance and reduced reliance on synthetic antibiotics. These effects are not isolated; they are interchained with broader ecosystem benefits. By modulating gut microbiota and reducing pathogen loads, chitosan indirectly improves water quality, minimizing organic waste and microbial contamination in aquatic environments.

Moreover, chitosan's biodegradability and biocompatibility ensure minimal ecological footprint, making it an ideal candidate for sustainable feed formulations, water treatments and therapeutic applications. Its ability to encapsulate bioactives, such as essential oils, metal nanoparticles, or phytochemicals, further expands its utility in precision aquaculture, offering targeted delivery and synergistic effects.

In summary, chitosan stands at the intersection of biotechnology, aquaculture innovation and circular economy principles. Its adoption not only enhances the resilience and productivity of aquaculture systems, but also contributes to a regenerative model of food production, where health, sustainability and resource circularity are mutually reinforcing.

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