Chitin and Chitosan in Aquaculture

Josafat Marina Ezquerra Brauer

Departamento de Investigación y Posgrado en Alimentos. Universidad de Sonora. Blvd. Luis Encinas y Rosales s/n, Col. Centro, 83000. Apdo. Postal 1658.

E-mail: ezquerra@guayacan.uson.mx

Abstract

Aquaculture is an important economic activity on many countries. However face several challenges mainly associated to feed and diseases development. Among the strategies applied to avoid or prevent those problems are the use of chitin and its derivate. Chitin consists of β-1-4-linked N-acetylglucosamine residues and is estimated as the second most important polysaccharide found in the nature. The main sources exploited are crustaceans. Chitin and chitosan are considerably versatile and promising biomaterials. The effect of chitin on several cultivated organism it was reported. Detecting that the inclusion of chitin in feed farmed organism improves not only the growth and feed conversion also stimulated the immune system against virus and protozoea by increasing the serum lysozyme; moreover the costs of production decrease. However, oversupplies of chitin in some fish species induce excessive deposition of fat liver, heart and carcass. Whereas chitosan, the deacetylated chitin derivate, considering more useful and interesting bioactive polymer, it was evaluated as encapsulated bioactive compounds during the culture of some farmed organisms. Chitosan encapsulated vitamin C, improve its liberation without lost its bioavailability. Although, chitosan can encapsulate antigen against white spot syndrome virus and vaccines, its efficacy depends of the virus. Moreover, chitosan posses properties, that may be useful to improve aquaculture wastewater quality. There are still many questions about the chitin and chitosan application in aquaculture, as chitosan encapsulated stability, the right chitin concentration according with the cultured specie, among others.

Keywords: Chitin, chitosan, aquaculture

Ezquerra, J. 2015. Chitin and Chitosan in Aquaculture. En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J., Rivas Vega, M. y Miranda Baeza, A. (Eds), Nutrición Acuícola: Investigación y Desarrollo, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México, ISBN 978-607-27-0593-7, pp. 220-233.

Resumen

La acuacultura es una actividad económica muy importante en varios países. Sin embargo enfrenta varios retos, la gran mayoría asociados a las dietas y al desarrollo de enfermedades. De las diversas estrategias seguidas para solventar algunos de los problemas antes mencionados están el uso de la quitina y quitosano. Se sabe que la quitina es uno de los polisacáridos más abundantes en la naturaleza, que está constituido por moléculas de N-acetil-D-glucosamina, con enlaces β-1-4, estimándose que es el segundo polisacárido más importante en la naturaleza. Siendo la principal fuente de este polímero los crustáceos. Tanto la quitina como el quitosano son considerados biomatariales muy versátiles y con aplicaciones promisorias. El efecto de la quitina en organismos cultivados ha sido reportado en varias especies. Detectándose que la inclusión de quitina en alimentos de organismos cultivados mejora no solo el crecimiento y la conversión alimenticia, sino que también estimula el sistema inmune de los organismos contra el ataque de virus y protozoea, incrementando la actividad de una enzima sérica, la lisosima, más aún los costos de producción disminuyen. Sin embargo, el uso de concentraciones elevadas de quitina en algunos peces puede inducir la acumulación excesiva de grasa en el hígado, corazón y desechos. Por otro lado, el quitosano, que es un derivado de la quitina deacetilada, es considerado un polímero bioactivo más útil, y ha sido evaluado para encapsular compuestos bioactivos durante el cultivo de algunos organismos. Al encapsular a la vitamina C con quitosano, se mejoró la liberación de la misma, sin menoscabo de su bioabilidad y aunque el qutiosano es capaz de encapsular antígenos contra el síndrome de la mancha blanca y vacunas, su eficacia dependerá del virus a atacar. Por otro lado, el quitosano posee propiedades que pueden ser útiles en mejorar la calidad del agua de cultivo. Aún hay muchas preguntas por resolver respecto a la aplicación de la quitina y el quitosano en acuacultura, como la estabilidad de los encapsulados, las concentraciones correctas de quitina acorde a la especie de cultivo, entre otras.

Ezquerra, J. 2015. Chitin and Chitosan in Aquaculture. En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J., Rivas Vega, M. y Miranda Baeza, A. (Eds), Nutrición Acuícola: Investigación y Desarrollo, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México, ISBN 978-607-27-0593-7, pp. 220-233.

Introduction

The aquaculture industry has increasingly attracted much attention for the intensive farming of fish and shellfish. However, fish and shrimp farming are facing problems such as bacterial diseases, farming environment, and feed contamination (García-Morales *et a*l., 2015). To address the aforementioned problems, the use of chitin and chitosan as a protective material appears to be a potential alternative.

Chitin is a naturally abundant mucopolysaccharide, which is found in shells or walls of invertebrates, fungi and yeasts. It is the main component of crustacean exoskeletons and is made up of calcium oxide and protein units (Muzzarelli, 1977). Chitin is well known to consist of 2-acetamido-2-deoxy- β -d-glucose through a β $(1 \rightarrow 4)$ linkage (Kumar, 2000). Chitin is a white, hard, inelastic, nitrogenous polysaccharide and the major source of surface pollution in coastal areas (Muzzarelli, 1973). Chitosan, an aminopolysaccharide, is prepared from shellfish chitin by treatment with alkali (Duta & Duta, 2004). Chitosan is the *N*-deacetylated derivative of chitin, although this *N*-deacetylation is almost never complete (Duta & Duta, 2004).

Chitin and its derivatives exhibit a variety of physicochemical and biological properties resulting in numerous applications in many industries, including aquaculture. In addition to its lack of toxicity and allergenicity, its biocompatibility, biodegradability and bioactivity make it a very attractive substance for diverse applications as a biomaterial, especially its novel application in the form of nanocarriers for bioactive compounds for aquaculture industry (Alishahi & Aïder, 2012). In the present study, the applications of chitin and chitosan in aquaculture field will be reviewed.

Chitin and Chitosan Structure

Chitin is a linear, highly crystalline homo polymer of β-1,4 N-acetyl glucosamine that are arranged in antiparallel (α), parallel (β) or mixed (γ) strands, with the (α)

Ezquerra, J. 2015. Chitin and Chitosan in Aquaculture. En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J., Rivas Vega, M. y Miranda Baeza, A. (Eds), Nutrición Acuícola: Investigación y Desarrollo, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México, ISBN 978-607-27-0593-7, pp. 220-233.

configuration being the most abundant (Figure 1) (Cheba, 2011). Whereas, chitosan usually refers to a family of polymers obtained after chitin deacetylation to varying degrees. In fact, the acetylation degree, which reflects the balance between the two types of residues (Figure 1), differentiates chitin from chitosan. When the DA (expressed as molar percentage) is lower than 50 mol%, the product is named chitosan and becomes soluble in acidic aqueous solutions (Kafetzopoulos *et al.*, 1993). During deacetylation, acetyl groups are removed but also depolymerization reaction occurs, indicated by changes in MW of chitosan. Chitin can be converted to chitosan by enzymatic preparations (Tokuyasu *et al*., 2000, Ilyina *et al*., 1999, Aiba, 1994) or chemical (Kurita *et al.*, 1977). Chemical methods are used extensively for commercial purpose of chitosan preparation because of their low cost and suitability to mass production (No & Meyers, 1995).

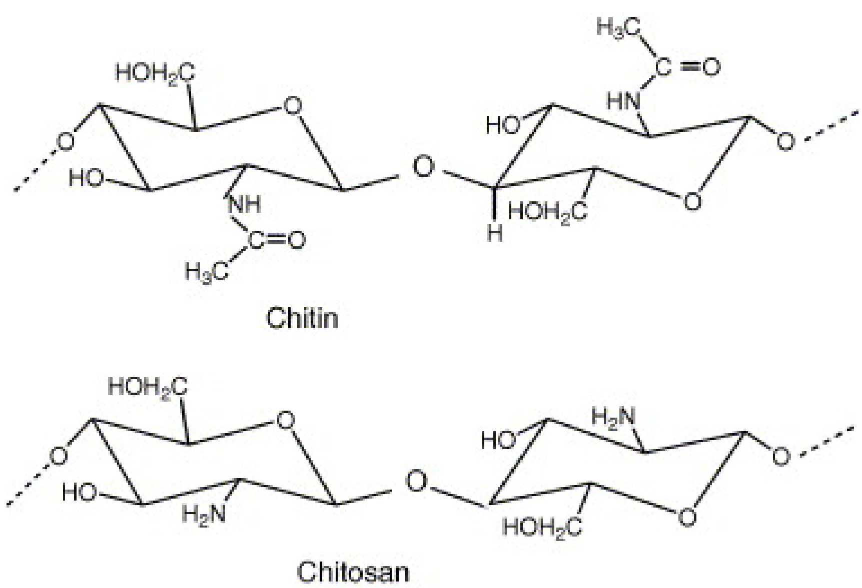

Figure 1. Chemical strucutres of chitin and chitosan. Source: Gamage & Shahidi, (2007)

Chitin and Chitosan Biological Properties

A review on the biological activities of chitin and chitosan such as: antibacterial, antifungal, antitumor and antioxidant, was recently published (Younes & Rinaudo, 2015). It has been demonstrated that chitin, like other polysaccharides derived from cellulose, has good film-forming properties and good stability promoted by the establishment of a hydrogen bond network between extended chains. Therefore, chitin gives original properties to the new materials due to its biocompatibility, biodegradability and non-toxicity, with antimicrobial activity and low immunogenicity (Younes & Rinaudo, 2015).

Chitosan and derivatives possess many beneficially properties such as biocompatibility, biodegradability, safety and also interesting biological activities, much attention has been paid to their applications especially in biomedical, food, biotechnology and pharmaceutical fields (Younes & Rinaudo, 2015). These properties are specially recognized in the field of food preservation and packaging to avoid the use of chemical preservatives and to produce edible antimicrobial films due to the good film forming properties of chitosan. Chitosan, as a polymeric ingredient with a good antimicrobial and antioxidant properties, does not migrate easily out of the protecting film and has better barrier properties (Alishahi & Aider, 2012). The use of chitosan in the aquaculture industry was also described in the review of Alishahi & Aider (2012).

Chitin and Chitosan Applications in Aquaculture Industry

Chitin and chitosan compounds had potential and versatile uses in the aquaculture industry. These applications are summarized in tables 1. Among their attractive biological activities as feed supplementation, encapsulation and effluent treatment will be discussed in detail below.

Table 1. Effect of the chitin and chitosan application on cultivated organisms

Organism	Diet inclusion results	References
Cobia (*Rachycentron canadum*)	Shrimp waste: costs of production decrease	Lu & Ku, 2012.
Dover sole *(Solea solea)*	Chitosan encapsulated vaccines: improve immune system against some virus	Tian *et al.*, 2008
European seabass *(Dicentrarchus labrax)*	Chitosan encapsulated vaccines against *Vibrio anguillarum*: lower protection	Rajeskumar *et al.,* 2008.
Gilt-hear bream (*Sparus aurata*)	Chitin: improve immune system against virus and protozoea	Cuesta *et al*., 2003
Olive flounder (*Paralichthys olivaceus*)	Chitosan-coated diet: increased non-specific immune response and improved the water quality in the fish tank	Cha et al., 2008
Rainbow trout (*Oncorhynchus mykiss*)	Chitosan encapsulated vitamin C: improve vitamin C liberation without biodisponibility loss	Alishahi *et al*., 2011
Rainbow trout (*Oncorhynchus mykiss*)	Chitosan: enhance the haematological parameters and resistance against some environmental stress	Meshkini *et al.*, 2012.
Rainbow trout (*Oncorhynchus mykiss*)	Chitin: increase serum lysozyme and improved immune system	Vahedi & Ghodratizadeh, 2011.
Tilapia (*Orechromis aerus*)	Diet containing ChiB565, improved growth and feed conversion compared with a control diet.	Zhan *et al.*, 2014

White shrimp (*Penaeus vannamei*)	Moderate chitosan concentration was beneficial to shrimp development	Niu *et al.*, 2011
White shrimp (*Penaeus vannamei*)	Chitosan encapsulated antigen against WSSV: improve protection	Rajeskumar *et al.*, 2009.

Feed supplementation

Chitin is an important food for fish, particularly during larval stages. Dietary chitin stimulates the innate immune response in gilthead sea bream (Esteban *et al.*, 2001) by increasing complement activity, cytotoxic activity, respiratory burst and phagocyte activity, but not lysozyme activity. Chitin in fish diets interferes with bacteriolytic activity of lysozyme in trout stomach (Lindsay, 1984). Thus, chitin may be of interest as an immunostimulant (Esteban *et al.*, 2001). The protective effects of chitin and chitosan by injection or immersion have been reported in rainbow trout, *Oncorhynchus mykiss*, against *Aeromonas salmonicida*and *Vibrio anguillarum* (Anderson *et al.*, 1995; Siwicki *et al.*, 1994; Sakai *et al.*, 1992), brook trout, *Salvelinus fontinalis*, against *A. salmonicida* (Anderson & Siwicky, 1994) and in yellowtail, *Seriola quinqueradiata*, against *Pasteurella piscicida* (Kawakami *et al.*, 1998). In contrast, Shiau & Yu (1999) reported that both chitin and chitosan supplementation depresses tilapia (*Oreoochromis niloticus*^{x}*O. aerus*) growth regardless of the supplementation level. Authors infer that the differences could be due to enzyme activity present in the organism studied.

There are several reports about the effects of dietary chitin and chitosan on crustacea. Deshimaru & Kuroki (1974) suggested that a dietary source of glucosamine is unnecessary for *Penaeus japonicus*. While Kitabayashi *et al.* (1971) demonstrated that the addition of glucosamine at 5.2 g/kg diet improved the growth of *P. japonicus*, but growth was retarded if chitin was added to the diet. Akiyama *et al.* (1992) recommended a minimum dietary level of chitin at 5 g/kg diet in shrimp feed. Wang & Chen (2005) established that fed *L. vannamei* with chitin at 6 µg/g or chitosan at 4 µg/g or less increased its immune ability and resistance to *V. alginolyticus* infection and Niu *et al.* (2011) reported that the level of chitosan supplemented in the postlarval *L. vannamei* diet should be between 2.13 and 2.67 g/kg to improve its growth and survival.

On the other hand, although the various enzymes and pathways for carbohydrates digestion have been detected in fish (Shimerno, 1974), they can develop signs of ill health if there is a high concentration of carbohydrate in their diet. The incorporation of inappropriate carbohydrate level in the diet has been identified to cause prolonged hyperglycemia (Hatlen *et al.*, 2005), fatty fish (Fernández *et al.*, 2007), liver dysfunction (Hilton & Atkinson, 1982), and impaired bone development (Tan *et al.*, 2007). Even though, if the carbohydrates are applied in the right concentration or presentation it can increase the development of the fish. Wang & Li (2010) detected that dietary chitosan nanoparticles supplementation improved, not only the growth performance of tilapia, also its meat quality.

Encapsulation

Vaccination plays a critical role in protecting commercially raised fish from bacterial, viral and parasitic diseases (Rivas-Aravena *et al.*, 2013). However, when incorporated into drug delivery systems, these bioactive components are often hydrolyzed by harsh conditions in the gastrointestinal tract (Alishahi *et al.*, 2011). It was stated that many oral delivery systems for bioactive aquaculture compounds meet three major barriers in passing through the gastrointestinal tract, namely enzymatic barriers from the host luminal and membrane bound enzymes, immunological cells present within both the enterocytes and underlying connective tissue and the physical barrier of the epithelial cells (Schep *et al.*, 1999). Then, the encapsulation of vaccines or any bioactive compounds could be a promising way to overcome these problems. Encapsulation is a process used to entrap active components and release them under controlled conditions (Deladino *et al.*, 2008).

The beneficial effects on farmed organism of chitosan as an encapsulating agent have been demonstrated.

Alisha *et al.* (2011) showed that the shelf life of vitamin C was increased in rainbow trout feed for 20 days, whereas the control, which was fed vitamin C alone, lost significant vitamin C content in a few days. The authors also showed that vitamin C was released in the gastrointestinal tract of rainbow trout in a controlled manner and that chitosan nanoparticles protected vitamin C from the harsh conditions of acidic and enzymatic hydrolysis in the gastrointestinal tract of the rainbow trout.

Concerning the antiviral activity of chitosan, it has been demonstrated that chitosan nanoparticles could be used to encapsulate DNA, which was then beneficially incorporated into shrimp feed to protect them from white spot syndrome virus (Rajeshkumar *et al.*, 2009). Incorporated chitosan nanoparticles containing a DNA vaccine into Asian sea bass (*Lates calcarifer*) feed, moderate protection against experimental *Vibrio anguillarum* infection was detected (Rajeshkumar *et al.,* 2008). In addition, chitosan microspheres loaded with plasmid vaccine were used to orally immunize Japanese flounder (*Paralichthys olivaceus*) (Tian *et al.*, 2008).

Despite the apparent advantages offered by chitosan, there have not been sufficient studies on the effectiveness of encapsulating antigens with chitosan.

Effluent treatment

During the culture of any organism a lot of organic compounds and inorganic nutrients are produced, inducing deterioration of receiving water quality. Chitosan served as an effective coagulating agent in removing proteins from wastewater as well as for removal of metal ions for industrial wastewater (Renault *et al.*, 2009; Gamage & Shahidi, 2007) therefore it has been used to improve aquaculture wastewater quality. The chitosan could selectively remove pathogens like *Edwarsiella ictaluri* (Chung, 2010). Also, it was detected that chitosan is an effective bio-flocculant for phytoplankton removal in outdoor shrimp culture tanks (Lertsutthiwong *et al.*, 2009). Furthermore, chitosan bead immobilized algae system with *Senedesmus sp* was efficient in removing phosphate and nitrate from water (Fierro *et al.*, 2008). Cha *et al.* (2008) reported that fed olive flounder with chitosan-coated moist pellet diet besides to increase non-specific immune response of the organism, the water quality of the fish tank was improved. Finally, it is important to notice that the efficacy of the chitosan to remove suspended solids, organic material and pathogens will depend on its deacetylation degree and the pH of the wastewater (Chung, 2010).

Conclusions

Chitin and chitosan has attracted a great deal of attention in the aquaculture industry due to its properties. Both compounds can be used as feed, feed supplementation or as a new vehicle for the improvement of the delivery of active compounds. Chitosan could remove suspended solids, organic compounds, and pathogens, and then it can be used to improve aquaculture wastewater quality. Chitin and mainly chitosan could be successfully be incorporated into diet aquaculture organisms like fish and shrimp, to improve the growth, health and meat quality of those culture organism. However, there are still many questions about the chitin and chitosan application in aquaculture, as chitosan encapsulated stability, the right chitin concentration according with the cultured specie, among others.

References

Aiba S.I. (1994) Preparation of N-acetylchitooligosaccharides by hydrolysis of chitosan with chitinase followed by N-acetylation. *Carbohydrate Research*, **265**, 323–328.

Akiyama D.M., Dominy W.G. & Lawrence A.L. (1992) Panaeid shrimp nutrition. In *Marine Shrimp Culture: Principles and Practice* (Fast, A.W. & Lester, L.J. eds), pp. 535-568. Elsevier Science Publishers, Amsterdam, the Netherlands.

Alishahi A. & Aïder M. (2012). Applications of chitosan in the seafod industry and aquaculture: A review. *Food Bioprocess Technology*, **5**, 817-830.

Alishahi A., Mirvaghefi A., Tehrani M. R., Farahmand H., Koshio, S., Dorkoosh F. A., *et al.* (2011). Chitosan nanoparticle to carry vitamin C through the gastrointestinal tract and induce the non- specific immunity system of rainbow trout (*Oncorhynchus mykiss*). *Carbohydrate Polymers*, **86**, 142-146.

Anderson D.P. & Siwicki A.K. (1994). Duration of protection against *Aeromonas salmonicida* in brook trout immunostimulated with glucan or chitosan by injection or immersion. *The Progressive Fish-Culturist*, **56**, 258‑261.

Anderson D.P., Siwicki A.K. & Rumsey G.L. (1995). Injection or immersion delivery of selected immunostimulants to trout demonstrate enhancement of nonspecific defense mechanisms and protective immunity. In *Diseases in Asian aquaculture, 11 fish health section* (Shariff M., Subasighe R.P., Arthur J.R. eds.), pp. 413‑426. Asian Fisheries Society, Manila, Philippines.

Cha S.H., Lee J.S., Song Ch.B., Lee K.J. & Jeon Y.J. (2008). Effects of chitosan-coated diet on improving wáter quality and innate immunity in the olive flounder, *Paralichthys olivaceus*. *Aquaculture*, **278**, 110-118.

Cheba B. A. (2011). Chitin and chitosan: Marine biopolymers with unique properties and versatile applications. *Global Journal of Biotechnology & Biochemistry* **6**, 149-153.

Chung, Y-C. (2010). Improvement of aquaculture wastewater using chitosan of different degrees of deacetylation. *Environmental Technology*, **27**, 119-1208.

Cuesta A., Esteban M.A. & Mesequer J. (2003). *In vivo* effect of chitin particles on the innate celular immune system of gilthead seabream (*Saparus aurata* L.). *Fish Shellfish Immunology*, **15**, 1-11.

Deladino L., Anbinder P. S., Navarro A. S., & Martino M. N. (2008). Encapsulation of natural antioxidants extracted from Ilex paraguariensis. *Carbohydrate Polymer*, **71**, 126-134.

Deshimaru O. & Kuroki K. (1974) Studies on a purified diet for prawn. I. Basal composition of diet. *Bulletin of the Japanse Society for the Science of Fish*, **40**, 413-419.

Duta P.K. & Duta J. (2004). Chitin and chitosan: Chemistry, properties and applications. *Journal of Scientific & Industrial Research*, **63**, 20-31.

Esteban M.A., Cuesta A., Ortuno J. & Meseguer J. (2001). Immunomodulatory effects of dietary intake of chitin on gilthead seabream (*Sparus aurata* L.) innate immune system. *Fish & Shellfish Immunology*, **11**, 303-315.

Fernández F., Miquel A.G., Cordoba M., Varas M., Meton I., Casera A. & Baanante V. (2007). Effects of diets with distintict protei-carbohydrate ratios on nutritient digestibililty, growth perfomance, body composition and liver intermediary enzyme activities in gilthead sea bream (*Sparus aurate* L.) fingerlings. *Journal of Experimentla Marine Biology and Ecology*, **343**, 1-10.

Fierro S., Sànchez-Saavedra M.P., Copalcúa C. (2008). Nitrate and phosphate removal by chitosan immobilized Senedsmus. *Bioresource Technology*, **99**, 1274-1279.

García-Morales M.H., Pérez-Velázquez M., González-Félix M.L., Burgos-Hernández A., Cortez-Rocha M.O., Bringas-Alvarado L. & Ezquerra-Brauer J.M. (2015). Effects of fumonisin B_1-containing feed on the muscle proteins and ice-storage life of white shrimp (*Litopenaeus vannamei*). *Journal of Aquatic Food Product Technology*, **24**, 340-353.

Gamage A. & Shahid F. (2007). Use of chitosan for the removal of metal ion contaminants and proteins from water. *Food Chemistry*, **104**, 989-996.

Hatlen B., Grisalde-Helland B. & Helland S.J. (2005). Growth, feed utilization adn body composition in two size groups of Atlantic halibut (*Hippooglossus hippoglossus*) fed diets differing in protein and carbohydrate content. *Aquaculture*, **249**, 401-408.

Hilton J.W. & Atkinson J.L. (1982). Response of rainbow trout to increased levels of availabel carbohydrate in practical diets. *Brithish Journal of Nutrition*, **47**, 597-607.

Ilyina A.V., Tatarinova N.Y. & Varlamov V.P. (1999) The preparation of low-molecular-weight chitosan using chitinolytic complex from *Streptomyces kurssanovii*. *Process Biochemistry*, **34**, 875–878.

Kafetzopoulos D., Martinou A. & Bouriotis V. (1993). Bioconversion of chitin to chitosan: Purification and characterization of chitin deacetylase from *Mucor rouxii*. *Proceedings of the National Academy of Sciences of the United States of America*, **90**, 2564-2568.

Kawakami H., Shinohara N.& Sakai M.(1998) The non-specific immunostimulation and adjuvant effects of *Vibrio anguillarum*bactewrin, M-glucan, chitin and Freund's complete adjuvant against *Pasteurella piscicida* infection in yellowtail. *Fish Pathology*, **33**, 287‑292.

Kitabayashi K., Kurata H. & Ishihawa S. (1971) Studies on formula feed for Kuruma prawn I. On the relationship among glucosamine, phosphorus, and calcium. *Bulletin of Tokai Regional Fisheries Research Laboratory*, **65**, 91-105.

Kumar MNVR. (2000). A review of chitin and chitosan applications. Reactive and *Functional Polymers*, **46**, 1-27.

Kurita K., Sannan T. & Iwakura Y. (1977). Studies on chitin, 4: Evidence for formation of block and random copolymers of N-acetyl-D-glucosamine and D-glucosamine by hetero- and homogeneous hydrolyses. *Macromolecular Chemistry and Pysics*, **178**, 3197-3202.

Lindsay G.J.H. (1984) Adsorption of rainbow trout (*Salmo gairdneri*) gastric lysozymes and chitinase by cellulose and chitin. *Aquaculture* **42**, 241-246.

Lu Ch-H. & Ku Ch-Ch. (2010). Effects of shrimp waste meal on growth performance and chtinase activity in juvenile cobia (*Rachycentron canadum*). *Aquaculture Research*, **44**, 1190-1195.

Luertsutthiwong P., Sutti S. & Powtongsook S. (2009). Optimization of chitosan flocculation for phytoplankton removal in shrimp culture ponds. *Aquaculture Engineering*, **41**, 188-193.

Meshkini S., Tafy A-A., Tukmechi A. & Farhang-Pajuh F. (2012). Effects of chitosan on hematological parameters and stress rsistance in rainbow trout (*Oncorhynchus myksiss*). *Veterinary Research Forum*, **3**, 49-54.

Muzzarelli R.A.A. (1977). Enzymatic synthesis of chitin and chitosan. Occurrence of chitin. In *Chitin* (Muzzarelli, R.A.A. eds.), pp. 5–44. Pregamon Press, New York, NY,

Muzzarelli R.A.A (1973). *Natural Chelating Polymers*, Pergamon Press, New York, p. 83.

Niu J. Liu Y.J., Lin H.Z., Mai K.S., Yang H.J., Liang G.Y. & Tian L.X. (2011). Effect of dietary chitosan on growth, survival and stress tolerance of postlarval shrimp, *Litopenaeus vannamei*. *Aquaculture Nutrition*, **17**, e406-e412.

No, H.K. & Meyers S.P. (1995). Preparation and characterization of chitin and chitosan-A review. *Journal Aquatic of Food Product and Technology*, **2**, 27–52.

Rajeshkumar S., Ishaq Ahmed V. D., Parameswaran V., Sudhakaran R., Sarath Babu V., & Sahl Hameed A. S. (2008). Potential use of chitosan nanoparticles for oral delivery of DNA vaccine in Asian sea bass (*Lates calcarifer*) to protect from *Vibrio anguillarum*. *Fish & Shellfish Immunology,* **25**, 47-56.

Rajeshkumar S., Venkatesan C., Sarathi M., Sarathbabu V., Thomas J., & Anver Basha, K. (2009). Oral delivery of DNA construct using chitosan nanoparticles to protect the shrimp from white spot syndrome virus (WSSV). *Fish & Shellfish Immunology*, **26**, 429-437.

Renault F., Sancey B., Badot P-M. & Crini G. (2009). Chitosan for coagulation/flocculation processes –An econfriendly approach. *European Polymer Journal*, **45**, 1337-1348.

Rivas-Aravena A., Sandino A.M. & Spencer e. (2013). Nanoparticles and microparticles of polymers and polysaccharides to administer fish vaccines. *Biological Research*, **46**, 407-419.

Roberts G.A.F (1992). Structure of chitin and chitosan. In *Chitin Chemistry* (Roberts G.A.E., ed.) pp. 85–91. Palgrave Macmillan: London, UK, 1992.

Sakai M., Kamiya H., Ishii S., Atsuta S. & Kobayashi M. (1992) The immunostimulating effects of chitin in rainbow trout, *Oncorhynchus mykiss*. In *Diseases in Asian aquaculture I,fFish health sectio*, Shariff M., Subasighe R.P., Arthur J.R. (Eds.). Asian Fisheries Society, Manila, Philippines, pp. 413–417.

Schep L. J., Tucker I. G., Young G., Ledger R., & Butt, A. G. (1999). Controlled release opportunities for oral peptide delivery in aquaculture. *Journal of Controlled Release*, **59**, 1-14.

Shiau S-Y. & Yu Y-P. (1999). Dietary supplementation of chitin and chitosan depresses growth in tilapia, *Oreochromis niloticus*×*O. aureus*. *Aquaculture*, **179**, 439-446.

Siwicki A.K., Anderson D.P.& Rumsey G.L. (1994) Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. *Veterinary Immunology and Immunopathology*, **41**, 125‑139.

Tan Q., Xie S., Zhu X., Lei W. & Yang Y. (2007). Effect of dietary carbohydrate-to-lipid ratios on growth and feed utilization in Chisese longsnout catfish (*Leiocassis longrisotris* Gunther). *Journal of Applied Ichthyology*, **23**, 605-610.

Tian J., Yu J., & Sun X. (2008). Chitosan microspheres as candidate plasmid vaccine carrier for oral immunization of Japanese flounder (*Paralichthys olivaceus*). *Veterinary Immunology and Immunopathology*, **126**, 220-229.

Tokuyasu K., Mitsutomi M., Yamaguchi I., Hayashi K. & Mori Y. (2000). Recognition of chitooligosaccharides and their N-acetyl groups by putative subsites of chitin deacetylase from a deuteromycete, Colletotrichum lindemuthianum. *Biochemistry*, **39**, 8837-8843.

Vahedi G. & Ghodratizadeh S. (2011). Effect of chitin supplemented diet on innate immune response of raibow trout. *World Journal of Fish and Marine Sciences*, **3**, 509-513.

Wang S.H. & Chen J.Ch. (2005). The protective effect of chitin and chitosan against *Vibrio alginolyticus* in White shrimp *Litopenaeus vannamei*. *Fish & Shellfish Immunology*, **19**, 191-204.

Wang Y. & Li J. (2010). Effects of chitosan nanoparticles on survival, growth and meat quality of tilapia, Orechromis nilotica. *Nanotoxicology*, **5**, 425-431.

Younes I. & Rinaudo M. (2015). Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Marine Drugs*, **13**, 1133-1174.

Zhan Y., Zhou Z., Liu Y., Cao Y., He S., Huo F., Quin C., Yao B & Ringo E. (2014). High-yield production of a chitinase from *Aeromonas veronii* B565 as a potential feed supplement for warm-water aquaculture. *Applied Microbiology Biotechnology*, **98**, 1651-1662.