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Review

Chitinase and Insect Meal in Aquaculture Nutrition: A Comprehensive Overview of the Latest Achievements

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Abstract: The aquaculture industry is looking for sustainable alternatives to conventional fish meals in fish feed, and insect-based meals are proving to be a promising solution. These meals are nutritionally optimal as they have a high protein content and an ideal amino acid profile. However, the presence of chitin, a component of the insect exoskeleton in these meals presents both an opportunity and a challenge. Chitosan, a derivative of chitin, is known to improve the physiological functions of fish, including growth, immunity, and disease resistance. While chitin and its derivative chitosan offer several physiological benefits, their presence can affect the digestibility of feed in some fish species, making the inclusion of insect-based meals in aquafeeds complex. While studies suggest positive effects, some problems, such as reduced growth rates in certain species, emphasize the need for further research on chitin digestion in fish. Chitinase, an enzyme that breaks down chitin, is being investigated as a potential solution to improve the nutritional value of insect meals in aquafeed. This review provides a comprehensive analysis of the applications, benefits, and challenges of using chitinase in aquaculture, highlighting the enzyme's role in improving feed digestibility, disease control, and environmental sustainability. Extensive research is required to fully understand the potential of chitinase enzymes in aquaculture and to optimize their applications in this dynamic field. Overall, this review provides insight into the evolving landscape of insect-based meals and the applications of chitinase enzymes within sustainable aquaculture practices.

Keywords: chitinase; insect meal; chitin degradation; fungal chitinase; aquaculture nutrition

Key Contribution: This review deals with the synergistic application of chitinase enzymes and insect meal in aquaculture nutrition. It highlights the latest advances and emphasizes the intricate interplay between chitinase specificity; enzyme optimization; and the sustainable use of insect-based protein sources.

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1. Introduction

The aquaculture industry is currently facing a crucial challenge: the search for sustainable and economically viable alternatives to conventional fishmeal (FM) in fish feeds. One promising solution on the horizon is the use of insect-based meals as an alternative protein source due to their optimal nutritional properties, especially their high protein content and ideal amino acid profile [1,2], but also due to their potential to meet the growing demand for alternative protein sources in aquaculture feeds. Their potential is further enhanced by recent advances in processing, economic viability, and scalability [3]. Insect meals (IMs) are increasingly proving their suitability as a partial or complete replacement for

FM in aquaculture feeds. Numerous feeding trials conducted by different research groups including ours [1,4–10] have produced promising results in this respect. These studies highlight the potential of IM as a sustainable and effective alternative to conventional FM in aquaculture feed formulations. In our recent studies, we have found encouraging results with the incorporation of black soldier fly (*Hermetia illucens*) and yellow mealworm (*Tenebrio molitor*) meals into the feeds of carnivorous marine and freshwater fish [6–11]. These promising results are an important step towards the use of IMs as a sustainable alternative in aquaculture feeds and offer the aquaculture industry a viable way to overcome the challenges of sustainability and economic feasibility.

The use of insect meal in aqua feed can vary depending on the specific circumstances and the type of fish. Research indicates that IM can replace a significant proportion of FM in aquaculture feeds. For example, mealworms and housefly larvae meals can replace up to 40–80% and 75% of FM in the diet of Nile tilapia/standard catfish, respectively [1]. The use of IM as a sustainable alternative to FM in aquaculture has also shown promising results [2]. However, some difficult issues still need to be resolved, including the cost and expansion of use [2]. Chitin, a major component of the insect exoskeleton, can pose a challenge for digestibility. Monitoring the chitin content in insect meal and its impact on nutrient digestibility is critical for determining appropriate incorporation rates.

Insects do indeed offer a unique nutritional profile for aquaculture feeds, and a notable component of them is chitin. Chitin is a polysaccharide, the second most abundant on earth after cellulose, consisting of N-acetyl-2-amino-2-deoxyglucose (GlcNAc) units linked by β-(1$\rightarrow$4) bonds [11–13]. Chitin and its deacetylated derivative chitosan represent a significant source of insoluble "animal" fibers that may have potential utility as functional ingredients, or bioactive compounds in aquaculture feeds [12,14,15]. This component adds an interesting dimension to the nutritional value of IMs, and ongoing research is investigating the various ways in which chitin can positively impact the health and performance of fish in aquaculture systems.

Numerous studies have shown that chitosan plays an important role in improving various physiological functions in fish. These effects include improved growth performance, enhanced immunity, and improved antimicrobial capabilities, which underlines the multiple benefits of chitosan in fish farming [12]. The inclusion of chitin and chitosan in fish feed has various positive effects on different fish species in aquaculture. These effects include increased growth rates, improved feed efficiency, and increased disease resistance in species such as rainbow trout, Nile tilapia, grey mullet, red sea bream, Japanese eel, and yellowtail [16–21]. While chitosan and chitin in IMs provide numerous benefits, it is important to recognize that the presence of chitin can be a limiting factor in the use of insect-based feeds in aquaculture. The presence of chitin in fish feed can reduce the digestibility of the feed, as most fish species cannot efficiently digest and absorb chitin, as found in the study by [22]. However, the ability of fish to digest chitin is controversial as other fish species, such as cod (*Gadus morhua*) [23], juvenile cobia (*Rachycentron canadum*) [24], channel rockcod (*Sebastolobus alascanus*), splitnose rockfish (*Sebastes diploproa*), and black cod (*Anoplopoma fimbria*) [25] have shown activity of chitinase, the enzyme responsible for the degradation of chitin. These differences in the ability of fish species to digest chitin make the incorporation of IMs into aquafeeds even more complex.

Similar to cellulose, chitin can potentially serve as a prebiotic in fish diets. This is because it can increase bacterial diversity in the gut by promoting the proliferation of beneficial and chitin-degrading bacteria. As a result, chitin can stimulate fermentation in the gut, leading to the production of essential short-chain fatty acids, with acetate, propionate, and butyrate being the main end products of bacterial fermentation. This mechanism contributes to the improvement of intestinal health and the general well-being of fish [22,26].

Numerous studies have shown that insect-derived ingredients can effectively influence the microbial communities in the fish gut. Feeding insects to fish often results in a remarkable increase in the abundance of bacterial families, such as Bacillaceae, Lactobacillaceae, and Actinobacteria [6,8–10,27–30]. Similarly, adding chitosan to the feed has been found to promote the growth of beneficial bacteria in the fish gut while reducing the presence of potentially harmful pathogens [16]. In contrast to the potential benefits of chitin in fish feed, some studies have reported a reduced growth rate in salmon fed a chitin-rich diet. It has been hypothesized that chitin may act as an energy reservoir when fish are unable to efficiently digest and utilize this polysaccharide, suggesting that improper chitin digestion may affect fish growth and overall performance [31,32].

Chitinase and IM are becoming important components in aquaculture nutrition. The unique properties of insects and their suitability for use in aquafeeds as a substitute for FM have become a focus of recent research in aquaculture [33]. The use of the enzyme chitinase could enable the inclusion of higher amounts of IM in aquaculture feeds and thus increase the usefulness of IM [34]. Chitinase is an enzyme that breaks down chitin, a major component of the insect exoskeleton, into digestible carbohydrates. This process can increase the nutritional value of IM and make it more digestible for aquatic species. These enzymes have been detected in the digestive tracts of a variety of fish species, as demonstrated by several studies [35–38]. Fish species in which these enzymes have been detected include rainbow trout (*Oncorhynchus mykiss*) [39]. The growth of rainbow trout fed diets containing different amounts of chitin and the relationship between chitinolytic enzymes and chitin digestibility showed that the growth was significantly reduced when the diets contained, 4, 10 and 25% chitin compared to that of the controls fed starchy diets. There was no difference in growth rate between control fish and fish fed 10% N-acetylglucosamine (GlcNAc). A relatively high chitinase activity was found in the stomachs and a relatively high chitobiase activity in the intestines. These enzyme activities were similar in all trout, regardless of the amount of chitin in their diet, except that chitobiase in the gut of fish fed a diet containing GlcNAc had higher activity levels than controls. The study also found that chitin was not significantly digested when fed at levels of 10 and 30% of the diet and that the presence of antibiotics or live chitinolytic bacteria (*Vibrio alginolyticus*) in the diet had no effect on the digestibility of chitin. This suggests an endogenous origin of the chitinolytic enzymes in the gastrointestinal tract of trout [39]. Most of these chitin-degrading enzymes are found in the gastrointestinal tract of fish and exhibit acid-resistant activities when it comes to the degradation of insoluble chitin substrates. This resistance to acidic conditions suggests that these enzymes have the potential to effectively digest chitin-rich feeds, as shown in the study by [36]. Other studies have suggested that chitinase activity in fish is not sufficient to digest chitin [40]. However, some studies have hypothesized that fish are able to digest chitin to some extent and that the chitin metabolites are absorbed, suggesting that chitin may act as both a nutrient source and an antinutrient in fish [39]. The primary function of fish chitinases appears to be to chemically disrupt the exoskeleton or other chitin-containing external structures of prey so that the internal nutrients can be reached by the enzymes or to prevent blockage of the gut by these structures [41].

Chitinase has potential applications in aquaculture, particularly in the conversion of chitin into a usable form and in aiding the digestion of chitin in fish. However, extensive research is still needed to fully understand the efficacy and potential applications in this area. With this in mind, we provide here an in-depth analysis of the potential applications, benefits, and challenges of using chitinase in aquaculture, focusing on its role in improving feed digestibility, disease control, and environmental sustainability.

2. Is Chitin Really a Problem in Aquaculture?

Chitin is a nitrogenous, water-insoluble polymer that accumulates in significant quantities in fishery waste due to the processing of crustaceans and molluscs [42]. Chitin is known to protect against bacterial infections and reduce the risk of viral infection in aquaculture crustaceans [43]. Recent studies have investigated the use of dietary chitin in Nile tilapia, and rainbow trout. The results of these studies suggest that chitin may serve as both a nutrient source and an antinutrient in both Nile tilapia and rainbow trout [44]. Indeed, black soldier fly larvae meal (BSFLM) has been successfully used as a feed additive in several fish species, including Atlantic salmon, Nile tilapia, and rainbow trout. However, other studies have found that growth performance was suppressed when BSFLM was fed to turbot and rainbow trout [44]. The use of chitin and krill in aquaculture has been shown to modulate the gut microbiota of fish and improve their immune system [15]. The use of chitin-rich seafood waste as a dietary supplement for various warm-water fish species has been reported to result in immunostimulatory effects and improved fish growth, depending on the species and growth stage [42]. Chitin and its derivatives, such as chitosan, have potential benefits for aquaculture. They can be used as feed additives to promote growth, improve feed conversion, and stimulate the immune system of farmed animals [45]. Chitin has been found to act as a prebiotic and modulate the microbial communities in the gut of fish, which can improve their immunity and disease resistance [11,46]. Some studies even suggest that chitin and its derivative chitosan can boost shrimp immunity, helping to prevent and fight infectious diseases [47]. Chitosan in particular has been shown to have antimicrobial, anti-inflammatory, and immunostimulatory effects, making it a promising immunostimulant for aquaculture [48]. In addition, the inclusion of chitin and chitosan in fish feed formulations can improve the digestibility, nutrient uptake, and overall productivity of fish in aquaculture [49].

However, there are still questions regarding the optimal concentration of chitin and chitosan for different species and the stability of chitosan encapsulation. For example, Atlantic cod and Atlantic halibut appear to be unaffected by up to 5% chitin addition to the diet, while a chitin level of less than 1% of the diet negatively affected the growth and nutrient digestibility of Atlantic salmon [31]. Numerous studies have investigated the effects of adding insects to fish feed, and some have reported reduced fish growth and protein and fat absorption in the gut. These negative results are often attributed to the chitin content in insects, although concrete evidence is often lacking [39,50,51]. The degradation of chitin in the digestive system requires the involvement of three specific enzymes: chitinase, chitobiase, and lysozyme. Both carnivorous and omnivorous fish species possess these enzymes, as studies by [24,39] show. The evidence suggests that although fish have the ability to digest chitin to some extent, the chitinase content in fish is not very high, and the utilization of chitin as an energy source may be limited. The role of chitin in fish diets and its potential as a nutrient source or antinutrient needs further investigation to determine its actual effect on fish performance and nutrient digestibility. Overall, the use of chitin in aquaculture has both potential benefits and drawbacks, and its effectiveness may depend on the fish species and growth stage, but further research is needed to fully understand its application and optimize its use.

3. Chitinases from Insects and Microbial Organisms

One of the main problems with the inclusion of chitin in feed is the need for secondary production of chitinase. In this sense, the production of different insects or microbial organisms should be investigated. Chitinases are not limited to fish or mammals but are found in a variety of organisms from different biological kingdoms. These enzymes have been detected in arthropods, molluscs, protozoa, nematodes, and coelenterates. In addition, numerous insect species, including *Drosophila melanogaster*, *Tribolium castaneum*, *Anopheles gambiae*, *Bombyx mori*, *Hyphantria cunea*, *Chironomus tentans*, *Spodoptera litura*, *Choristoneura fumiferana*, *Helicoverpa armigera*, *Aedes aegypti*, *Culex quinquefasciatus*, *Lacanobia oleracea*, *Spodoptera exigua*, *Mamestra brassicae*, *Ostrinia furnacalis*, *and Acyrthosiphon pisum*, are known to produce chitinases [52,53]. This wide distribution of chitinases in different organisms underlines the importance of chitin degradation and utilization in nature. Indeed, chitinase genes are not only found in various insect species, but also in multiple copies in certain mosquito species, including *Anopheles gambiae*, *Aedes aegypti*, and *Culex quinquefasciatus* [52]. The first full-length cDNA of a chitinase gene was successfully cloned from *Manduca sexta* [54], representing an important milestone in the study of these enzymes. In addition, chitinase genes have been isolated from various crustaceans, extending their occurrence to marine organisms. These crustaceans include the Pacific white shrimp

(*Litopenaeus vannamei*), the Chinese mitten crab (*Eriocheir sinensis*), the black tiger shrimp (*Penaeus monodon*), the Ridge Tail prawn (*Exopalaemon carinicauda*), and the Kuruma shrimp (*Marsupenaeus japonicus*). This diversity of chitinase genes in different species reflects their importance for chitin metabolism in different biological contexts [55]. Chitinases are not only found in animals but are also produced by a variety of microorganisms and plants. Various microorganisms, including bacteria, actinomycetes, and fungi, have been found to secrete chitinases. Several bacterial genera, such as *Bacillus*, *Streptomyces*, *Brevibacillus*, *Serratia*, and *Chromobacterium*, are known for their ability to produce chitinases [56]. Similarly, numerous genera of fungi, including *Aspergillus*, *Trichoderma*, *Neurospora*, *Mucor*, *Lycoperdon*, *Metarhizium*, *Beauveria*, and *Lecanicillium*, are known for their ability to produce chitinases [57].

4. Fungal Chitinases: A Key to Improved Digestibility

The extraction of chitinases from other insects or microbial organisms is a promising way to enable the use of chitinases in fish feed. Fungi are the dominant group of chitinase producers among microorganisms, and many efficient chitinolytic fungi with potential applications have been identified in a variety of environments, including soil, water, marine litter, and plants. This suggests that fungal chitinases may be a sustainable option for chitin degradation in aquaculture [58]. Recent research has shown that the use of fungal chitinases in IM containing feeds may be a promising strategy to improve nutrient uptake in marine fish such as European seabass (*Dicentrarchus labrax*), which is one of the most commonly farmed species in Mediterranean aquaculture [59,60].

Aeromonas veronii B565, a bacterium isolated from the sediment of aquaculture ponds, produces chitinases, enzymes that can degrade chitin, a component of the cell walls of fungi and the exoskeletons of insects and crustaceans. These chitinases have been proposed as a potential means of controlling Myxozoa-related or fungal diseases in fish [61–63]. Myxozoa are a class of microscopic parasites that can cause severe damage in aquaculture, resulting in significant economic losses. Currently, there are no effective treatments for Myxozoa infections, so the development of new control strategies is a high priority [62].

Chitinolytic bacteria capable of producing chitinases were isolated from the gut microbiota of European seabass fed with different IMs [59]. These bacteria can help to degrade chitin, a major component of the exoskeleton of many marine organisms, which is generally indigestible for several economically valuable fish species [24,59]. This may improve the use of feeds containing a high proportion of chitin-rich IM. The degradation of chitin by chitinases can improve nutrient uptake in seabass. By degrading chitin, chitinases can improve feed digestibility and facilitate the access of digestive enzymes to entrapped proteins or lipids, thereby improving overall digestive health and nutrient absorption in seabass [59,60].

Chitin derivatives produced by chitinase-producing bacteria have been shown to modulate the immunological response of fish and improve disease resistance [60]. In particular, the study showed that red blood cell and white blood cell counts increased significantly in sea bass fed chitin derivatives, indicating an improved immune response. Bioconversion of chitin waste by *Stenotrophomonas maltophilia* to produce chitin derivatives has been shown to have a significant effect on the non-specific immune response of seabass [60]. In addition, fungal polysaccharides, which include chitin, can regulate the growth and immune response of fish, enhancing immune response and disease resistance [64]. This suggests that chitin derivatives produced by fungal chitinases may have immunomodulatory effects in fish.

A study on juvenile cobia (*Rachycentron canadum*) found that high levels of chitinase in the digestive tract are associated with fish that lack the mechanical structures to break down chitinous material [24]. This suggests that the use of fungal chitinases in fish feed may be particularly beneficial for species with limited ability to digest chitin. The use of fungal chitinases in fish feed containing IM has the potential to improve nutrient uptake and increase the nutritional value of the feed for marine fish, such as sea bass. Several fungal chitinases have been already investigated for use in aquaculture (Table 1).

Table 1. Studies evaluating the effects of different fungal chitinases used in aquaculture.

Fungal Chitinases	Major Finding(s)	References
Aspergillus flavus	Fungal chitinases from *Aspergillus flavus* have been shown to have an anti-fungal effect against many plant pathogenic fungi. This indicates that *Aspergillus flavus* chitinases can be used to control pests and diseases in aquaculture.	[65]
Mucor	Chitin-degrading fungi such as Mucor have been identified in the aquatic environment. This indicates that Mucor chitinases can be used for chitin degradation in aquaculture.	[66]
Trichoderma	Fungal chitinases from Trichoderma facilitate mycoparasitism of other fungi. This suggests that *Trichoderma* chitinases can be used to control fungal infections in aquaculture.	[58]
Aspergillus sp. S1–13	*Aspergillus* sp. S1–13 has been shown to synthesize chitinolytic enzymes when grown on a medium containing shrimp waste. This suggests that the chitinases of *Aspergillus* sp. S1–13 can be used to convert chitin waste from fisheries into simpler, useful components to reduce water pollution.	[66]

Fungal chitinases can help break down chitin, a major component of the exoskeleton of many marine organisms. This process can improve nutrient uptake in fish, making feed more efficient and beneficial for the fish [65,66]. Chitinases from fungi have been shown to have immunomodulatory effects. For example, fungal polysaccharides, which include chitin, can regulate the growth and immune response of fish, enhancing immune response and disease resistance [64]. Fungi are the dominant group of chitinase producers among microorganisms, and many efficient chitinolytic fungi with potential applications have been identified in a variety of environments, including soil, water, marine litter, and plants [65]. This suggests that fungal chitinases may be a sustainable option for mediating chitin degradation in aquaculture. In addition, fungal chitinases can be used to convert chitin-containing waste from fisheries into simpler, useful components, thereby reducing water pollution and contributing to waste management in aquaculture [67].

5. Enhanced Nutrient Utilization with Chitinase

The enzyme chitinase hydrolyses chitin into digestible carbohydrates that can be used in fish feed, thus increasing the nutritional value of the feed [63,68]. One of the ways in which chitinase improves nutrient uptake is by breaking down chitin-containing biomass, such as the waste of marine organisms, into simpler, useful components [68]. Once chitin is broken down into simpler oligosaccharides, these molecules become more bioavailable. This means that they can be more easily absorbed in the animal's digestive tract, contributing to overall nutrient uptake. This process effectively converts chitin waste into a usable form that can be added to fish feed, contributing to improved nutrient utilization [44].

Preliminary studies have shown that chitin oligosaccharides, products of chitinase activity, could serve as prebiotics. They may promote the growth of beneficial gut bacteria, which in turn play a role in nutrient absorption and overall gut health. Studies have shown that dietary chitin or its derivative chitosan acts as a prebiotic, modulating microbial communities in the gut of fish [11]. This modulation can lead to improved nutrient uptake and the general health of the fish. In addition, chitinase can be used in the biocontrol of pathogenic fungi and harmful insects as it acts on chitin, a major component of their structures. This function can contribute to disease management in aquaculture as it can help in the control of pathogenic fungi and harmful insects that have chitin structures [44].

Furthermore, the introduction of chitinase into the feed of fish can lead to an increase in chitin digestibility and consequently to an improvement in nutrient digestibility. Studies have shown that the inclusion of chitinase in the diet can improve chitin digestibility in fish such as juvenile cobia and Nile tilapia, resulting in higher growth rates and more efficient nutrient utilization [24,34,44]. The impact on aquaculture productivity is significant. By improving nutrient utilization, chitinase can contribute to the growth and health of aquatic organisms, leading to higher productivity in aquaculture. However, it is important to note that the digestibility of chitin may decrease with higher levels of chitin in the diet [44]. Therefore, while the inclusion of chitinase in the diet may improve digestibility to some extent, the specific increase to be expected may vary depending on chitin intake and fish species.

By adding chitinase, chitin-rich feed ingredients can be utilized more effectively. This can lead to a reduction in feed waste and improved feed conversion, which in turn leads to more sustainable and cost-effective farming. Improved nutrient absorption often correlates with better overall health. Furthermore, the use of chitinase can help to avoid chitin waste, which is a significant environmental problem in aquaculture. Despite these potential benefits, it is difficult to increase microbial chitinase production due to the inducibility of the enzyme, low titer, high production costs, and susceptibility to challenging environmental conditions. Therefore, further research is needed to optimize the production and application of chitinase in aquaculture [68].

6. Role of Chitinase in Disease Prevention and Immunity

Chitin in insect meal can complicate feeding in aquaculture but chitinase can play a crucial role in alleviating these feeding problems. Chitinases and chitin have been reported to be involved in host immune responses against microbes in a variety of organisms, including fish [69]. In aquaculture, chitinases may play an important role in disease prevention and boosting the immune system, especially when used in conjunction with IM. IM is a rich source of chitin, and chitinases can help to break down the chitin in the feed, thereby improving the nutrient uptake of the fish and enhancing their immune response [69,70]. Chitinases can improve the immune response of aquatic animals by degrading the chitin coat of pathogens. This can contribute to the prevention and control of infectious diseases in aquatic animals. Chitinases can increase the resistance of aquatic animals to various pathogens. For example, chitinases in crustaceans, such as *Procambarus clarkii* play an important role in animal immunity [69]. There are few studies on the long-term effects of chitinase supplementation on aquatic animals. Most studies have focused on the short-term effects of chitinase supplementation on growth, survival, and immune response. A study on juvenile *Penaeus monodon* found that increasing the amount of chitin in the feed had no significant effects on individual weight gain, specific growth rate, feed conversion ratio, production, or survival rate [71]. In another study with seabass, chitinase supplementation was found to improve some innate immune functions, including the expression of certain immune-related genes [58]. However, the study did not examine the long-term effects of chitinase supplementation. For fish to utilize chitin, chitinases must be present in the digestive tract [44]. Previous studies have demonstrated the presence of chitinases in the intestinal tract of fish, suggesting that fish can digest chitin and utilize it as a nutrient source [44,72]. The digestibility of chitin and intestinal enzyme activity of Nile tilapia and rainbow trout fed different levels of inclusion of BSFLM in the feed have been studied. The study found that both Nile tilapia and rainbow trout can digest chitin and could potentially utilize chitin as a nutrient source. However, their ability to digest chitin decreased as the inclusion level of chitin in the diet increased [44].

7. Inclusion of Chitin and Chitinase in Aquaculture Feed

Chitinase is known for its ability to cleave chitin into various chitin oligosaccharides, including oligomers, chitobiose, and N-acetyl-glucosamine. These chitin derivatives can exert various biological effects, e.g., acting as antibacterial agents, as triggers for the production of lysozyme, and as immunostimulators [73]. The optimal amount of chitinase that should be added to aquaculture feed may depend on various factors, such as the type of aquatic animal, the type of feed, and the desired outcome. The strain *Aeromonas veronii* B565, isolated from aquaculture tank sediment, has been shown to be capable of producing chitinases that can be used to control fish diseases associated with Myxozoa or fungal infections [63]. The chitinase obtained from *A. veronii* B565 has shown high specific exochitinase activity on colloidal chitin and has shown promise as an enzyme for aquafeed additives [63]. In one experiment, Nile tilapia (*Oreochromis niloticus*) were fed a diet containing 5% shrimp bran and enriched with 16.2 U/kg chitinase from *Aeromonas veronii*, specifically "ChiB565". This supplement had significant positive effects on the growth, feed conversion, and nitrogen digestibility of tilapia and even reduced the expression of il-1β (interleukin-1 beta) in the gut compared to a control diet without ChiB565. Remarkably, these protective effects of the ChiB565-containing diet were also observed for a period of 2 to 3 days after exposure to the pathogenic bacterium *Aeromonas hydrophila* [63]. While there is a proposed link between dietary chitinase and defense against chitin-containing pathogens [74], the specific effects of dietary chitinase on the blood and various immune tissues require further in-depth research and investigation.

Another study investigated the effects of chitosan supplementation on farmed fish and found that chitosan at moderate concentrations (4 and 7.5 g kg^{-1} feed) is recommended for the best growth performance [12]. A more recent study investigated the potential of shrimp waste meal and insect exuviae as sustainable chitin sources for fish feed and found that supplementing feed with chitin and chitosan increased growth rates and feed efficiency and improved disease resistance in several fish species [11]. However, the study did not investigate the optimal amount of chitinase to supplement aquaculture feed. A study on chitin digestibility and intestinal enzyme activity in Nile tilapia and rainbow trout found that both species can digest chitin and could potentially utilize chitin as a nutrient source, but their ability to digest chitin decreased with higher chitin inclusion level in the feed [44]. In a study conducted by [75], it was reported that the inclusion of 1.0% chitin and chitosan in the diet significantly improved the hematological parameters of kelp grouper (*Epinephelus bruneus*). These parameters included an increase in red blood cells, white blood cells, hemoglobin levels, lymphocytes, monocytes, and neutrophils. In addition, the enhanced immune response from chitin and chitosan supplementation contributed to increased disease resistance against the protozoan parasite *Philasterides dicentrarchi*. These results emphasize the potential immunostimulatory and protective effects of chitin and chitosan in the context of aquaculture and fish health. The recommended levels of chitin and chitinase in aquaculture feed may depend on fish species, age, and other factors. The effect of dietary chitin supplementation on survival and immunoreactivity of the shore crab, *Carcinus maenas*, was investigated by supplementing a fish-based diet with 0, 5, or 10% chitin for 11 weeks [43]. In a recent study, two types of BSF meals were prepared using BSF larvae reared either on vegetable substrates (VGS) or on fish offal substrates (FOS), respectively. Two doses of commercial chitinase from *Aspergillus niger* (2 g/kg and 5 g/kg of feed) were added to the diets containing BSF/VGS or BSF/FOS larval meals. The study investigated the effect of chitinase supplementation on gut histopathology and immune responses following *Escherichia coli* lipopolysaccharide challenge in Nile tilapia fed chitinase-enriched BSFLM [61]. The addition of chitinase led to changes in gut histopathology and immune responses in fish. Chitinase supplementation increased the expression of tlr2, il-1β, and il-6 genes in the head kidney of fish fed BSF/VGS compared to fish fed the control diets. High doses of chitinase decreased the expressions of the tlr5 gene in the spleen and the mhcII-α gene in the head kidney of fish fed FOD5 feed compared to fish fed FOD0 diet [61]. The addition of 5 g/kg chitinase to a long chain polyunsaturated fatty acid-enriched BSF-based feed improved growth and prevented intestinal inflammation in Atlantic salmon [76]. This suggests that the inclusion of chitinase in aquaculture feeds could enhance the digestibility of chitin and improve the utilization of insect meal as a sustainable protein source for fish.

8. Latest Technological Advances in Chitinase Production for Aquaculture

The production of chitinase for commercial aquaculture operations usually involves microbial fermentation and enzyme purification processes. Microbial fermentation is a key process for the commercial production of chitinase enzymes for aquaculture applications. This process harnesses the productive chitinase-producing capabilities of certain microorganisms, both bacteria and fungi, and is instrumental in meeting the aquaculture industry's growing demands for sustainable disease management and improved feed efficiency.

Bacterial fermentation is one of the most important ways to produce chitinase for commercial aquaculture operations. Chitinase-producing bacteria, such as Bacillus and Pseudomonas strains, are cultured in specialized bioreactors to produce large quantities of chitinase. Bacillus spp. producing chitinases have been used as post-harvest biocontrol agents [56]. *Bacillus subtilis* isolates with chitinase activity can be cultured in bioreactors to produce chitinase. *Pseudomonas* spp. are known to produce chitinase and can be cultured in bioreactors to produce chitinase [77]. *Paenibacillus* sp. BISR-047 is a newly isolated thermotolerant bacterial strain that can produce chitinase [78]. *Myxococcus fulvus* UM01 is a novel myxobacterial strain that can produce large amounts of chitinase within a short period of time [79]. This approach offers several advantages, including the possibility of genetically manipulating bacteria, which can increase the efficiency of chitinase production.

In parallel, fungal fermentation has proven to be another robust strategy for chitinase production in aquaculture. Chitinase-producing fungi, including species of *Trichoderma* and *Aspergillus*, are cultivated under strictly regulated conditions to promote the secretion of chitinase enzymes. A marine soil isolate *Aspergillus terreus*, which has chitinase activity, was cultured on a medium of sucrose, peptone, and yeast extract. Colloidal chitin replaced sucrose as a carbon source to increase chitinase production [80]. A biocontrol fungus, *Trichoderma asperellum* PQ34, produces extracellular chitinase with antifungal activity [81]. A dose of 40 U/mL chitinase inhibited the growth of fungi after 96 h of treatment in mango and chili fruit infected with anthracnose. *Trichoderma koningiopsis* UFSMQ40 produces chitinase via solid-phase fermentation [82]. This fungus was previously isolated from bed bugs of *Tibraca limbativentris* Stal (Hemiptera: Pentatomidae) and selected from 51 isolated fungal strains. *Aspergillus niger* LOCK 62 produces antifungal chitinase, which can be used in biological control [83]. Chitinolytic enzymes are able to dissolve the cell walls of many fungi.

Genetic engineering is a cutting-edge approach that offers enormous potential for improving chitinase production in aquaculture. By modifying microorganisms through genetic manipulation, researchers and biotechnologists can increase chitinase yields, optimize enzyme properties, and contribute to the sustainability and efficiency of aquaculture operations. The genes for chitin metabolism in Atlantic salmon play a role in the development and maintenance of a chitin-based barrier in the fish [84]. Genetic engineering can be used to increase the chitinase yield and optimize the enzyme properties in Atlantic salmon. *Achromobacter xylosoxidans*, isolated from shrimp waste, can produce chitinase by genetic engineering [60]. This can be a cost-effective and environmentally friendly way to produce chitinase. The chitinase gene Ch-chit is involved in the regulation of biomineralization in *Crassostrea hongkongensis* [85]. With the help of genetic engineering, the chitinase yield can be increased and the enzyme properties of *Paenibacillus* sp. can be optimized [86,87]. This can be a cost-effective and environmentally friendly way to produce chitinase.

Yeast expression systems, such as *Pichia pastoris*, offer an attractive platform for the genetic modification of microorganisms for the expression and secretion of chitinase enzymes. *Pichia pastoris* is a popular yeast expression system for the production of recombinant proteins, including chitinase [88,89]. A polycistronic expression vector containing the genes of chitin deacetylase ChDaII, chitinase from *Thermomyces lanuginosus*, and chitosanases from *Aspergillus fumigatus* was expressed under the control of the same promoter in the methylotrophic yeast *Pichia pastoris* and characterized for their synergistic effects on their respective substrates [88]. *Saccharomyces cerevisiae* is another yeast expression system that can be used for the production of chitinase [90]. *Saccharomyces cerevisiae* has two genes coding for chitinase, CTS1 and CTS2. *Aspergillus niger* is a fungus that produces antifungal chitinase, which can be used in biological control [83]. This fungus can be genetically engineered to increase the yield of chitinase and optimize the enzyme properties. These genetic engineering strategies are powerful tools to revolutionize chitinase production in aquaculture. They offer the potential not only to increase chitinase yield but also to tailor the properties of the enzyme to specific aquaculture applications.

However, it must be ensured that genetically modified microorganisms comply with safety and regulatory requirements to ensure responsible use of these innovative approaches in aquaculture. Furthermore, as the aquaculture industry continues to expand and diversify, there is a constant need for innovation in fermentation technologies to improve the cost-efficiency and scalability of chitinase production, contributing to the sustainable growth of this important sector.

9. Challenges in Using Chitinase in Aquaculture

Chitinases have different sources, properties, and mechanisms of action that seem to complicate optimization procedures and make standardization techniques for improved practical applications complex [91]. This diversity can complicate the development and utilization of chitinases in industrial and practical applications. While some fish species can digest chitin, others may not be able to digest it effectively [24]. This may limit the use of chitin as a nutrient source in aquaculture.

There are several challenges associated with the use of chitinase in IM. One of the main challenges is the specificity of the chitinase enzymes for certain types of chitin structures, which may be different for different insect species [91,92]. Selecting a chitinase that effectively degrades chitin in the exoskeletons of the targeted insect species is a significant challenge [91]. These challenges arise from the complex nature of chitin and the variability of chitinase enzymes, as well as specific IM processing considerations. Insect species used for IM production may have different chitin compositions in their exoskeletons, making it difficult to develop a universal chitinase treatment that efficiently degrades chitin in all cases [93,94]. The exoskeleton of insects is mainly composed of chitin, and the regular moulting process is completed by the synergistic action of different enzymes involved in chitin synthesis and degradation [92]. Chitinase enzymes are able to degrade chitin, which is found in the cell walls of fungi and also in the exoskeletons of insects [66]. However, chitinase enzymes may have specificity for certain types of chitin structures, and their efficacy may vary in different insect species [92,93]. Therefore, the selection of a chitinase that effectively degrades the chitin in the exoskeletons of the targeted insect species remains a major challenge.

Determining the optimal conditions for chitinase treatment in IM processing is critical, but these conditions may need to be adjusted depending on the specific insect species and the desired properties of the IM [93,95,96]. The effectiveness of chitinase is influenced by environmental factors such as pH, temperature, and reaction time [93,96]. Studies have shown that the optimal conditions for chitinase treatment can vary depending on the insect species and chitin source. For example, a study on *Trichoderma virens* found that the optimal conditions for chitinase production were an incubation period of 7 days, a temperature of 30 °C, and a substrate humidity of 70% [96]. Another study with *Chitiolyticbacter meiyuanensis* SYBC-H1 found that the optimal conditions for chitinase production were a pH of 7.0, a temperature of 30 °C, and a substrate concentration of 1.5% [95]. Insect chitinases have theoretical molecular masses between 40 kDa and 85 kDa and also differ in terms of their pH optima (pH 4–8) and isoelectric points (pH 5–7) [93]. Therefore, it is important to consider the optimal pH range for the chitinase enzyme used in IM processing.

Chitinase treatment during the processing of IM can affect the nutritional composition of the meal, especially the essential nutrients such as proteins and lipids. It is, therefore, important to ensure that the treatment process preserves these nutrients while effectively degrading the chitin. This requires careful optimization of processing conditions, including pH, temperature, and reaction time [92,97]. The cost of producing chitinase can be high, which may limit its use in aquaculture [15]. Finding a cost-effective method to produce chitinase is important for the industry. Chitinase treatment from lab-scale experiments to large-scale IM production involves several challenges related to scalability, cost-efficiency, and maintaining consistent quality across batches [65,98]. In large-scale chitinase production, the substrate chitin is pre-treated to improve its accessibility to the chitinase, which contributes to a higher efficiency of the process [65]. Optimizing the processing conditions for chitinase treatment is also crucial for large-scale production. Studies have shown that the optimal conditions for chitinase treatment can vary depending on the insect species and chitin source [95]. Therefore, it is important to carefully optimize processing conditions to ensure consistent quality across batches. In addition to optimizing the processing conditions, it is also important to consider the cost-effectiveness of chitinase treatment. The cost of chitinase production and the availability of chitinase enzymes can influence the overall cost of IM production [98]. The profitability of chitinase in aquaculture may not be universally available due to the different conditions and contexts of the various studies. It is important to note that the field of chitinase application in aquaculture is dynamic and there may have been recent advances. Ongoing research and industry developments will continue to shape the understanding of the commercial viability of chitinase in aquaculture.

Overall, ongoing research and technological advances aim to overcome the challenges associated with chitinase treatment in IM processing and ensure the sustainable and effective use of IM in various applications, including aquaculture feed.

10. Conclusions and Future Perspectives

As the aquaculture industry actively seeks environmentally friendly and economically viable solutions, understanding the interplay between insect-based meals, chitin, and chitosan is critical. The potential benefits, including improved growth and increased disease resistance, must be weighed against the challenges associated with diet digestibility in certain fish species.

In addition, exploring chitinase as a potential solution to improve the nutritional value of IMs opens up new avenues for research and application. To realize the full potential of this enzyme, a comprehensive understanding, rigorous research, and strategic optimization of chitinases in aquaculture are required. This review provides researchers, practitioners, and stakeholders alike with valuable insights into the evolving landscape of insect-based meals and their applications. It addresses the complexity of chitin and chitosan and explores the role of chitinases. In doing so, it contributes to the pursuit of sustainable and efficient aquaculture practices.

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