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PROTEASE INHIBITORS IN FISH FEED: CHALLENGES AND STRATEGIES FOR DETOXIFICATION

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The increasing replacement of fishmeal with plant-derived ingredients in aquafeeds has raised concerns about the presence of anti-nutritional factors, among which protease inhibitors (PIs) are particularly significant. Found abundantly in soybean meal, groundnut meal, and other legumes, protease inhibitors interfere with digestive enzymes such as trypsin and chymotrypsin, thereby reducing protein digestibility, amino acid availability, and overall feed efficiency. Their action often results in impaired growth, pancreatic hypertrophy, altered gut morphology, and compromised health in sensitive fish species. The challenges posed by protease inhibitors are further amplified when plant protein inclusion levels are high, especially in carnivorous species with limited tolerance. Strategies to mitigate these effects include thermal processing (toasting, extrusion, autoclaving), fermentation, enzyme supplementation with exogenous proteases, genetic selection of low-PI crop varieties, and optimized feed formulations combining plant and animal protein sources. Recent advances in biotechnological approaches and enzyme supplementation have shown promise in reducing PI-related effects while supporting sustainable aquaculture. This review highlights the sources, mechanisms, and impacts of protease inhibitors in fish nutrition, and discusses current and emerging strategies for their detoxification to ensure efficient and sustainable fish feed formulation

The rapid expansion of aquaculture as the fastest-growing food production sector has significantly increased the demand for nutritionally balanced and cost-effective fish feeds. Traditionally, fishmeal has been the primary protein source in aquafeeds due to its high digestibility and balanced amino acid profile. However, sustainability concerns, rising costs, and limited availability of fishmeal have driven the industry toward greater reliance on plant-derived proteins such as soybean meal, groundnut meal, lupin seed, peas, and other legumes (Krogdahl et al., 2010; Francis et al., 2001).

Despite their advantages, plant ingredients are often limited by the presence of anti-nutritional factors (ANFs), which interfere with nutrient utilization and fish health. Among these, protease inhibitors (PIs)—including trypsin inhibitors (TI) and chymotrypsin inhibitors (CI)—are particularly important because they directly affect protein digestion, the most critical nutrient for fish growth and metabolism. By binding to digestive enzymes in the gastrointestinal tract, PIs reduce protein hydrolysis, leading to poor amino acid availability, pancreatic stress, impaired growth, and overall reduced feed efficiency.

Given the increasing use of plant proteins in aquafeeds, understanding the role, mechanisms, and consequences of dietary protease inhibitors is vital. Equally important are strategies to mitigate their negative effects through processing, enzymatic supplementation, biotechnological innovations, and feed formulation approaches. This review highlights the sources, mechanisms of action, health impacts, challenges, and recent advances related to protease inhibitors in fish feed, with a focus on practical solutions to improve fish performance and ensure the sustainability of aquaculture.

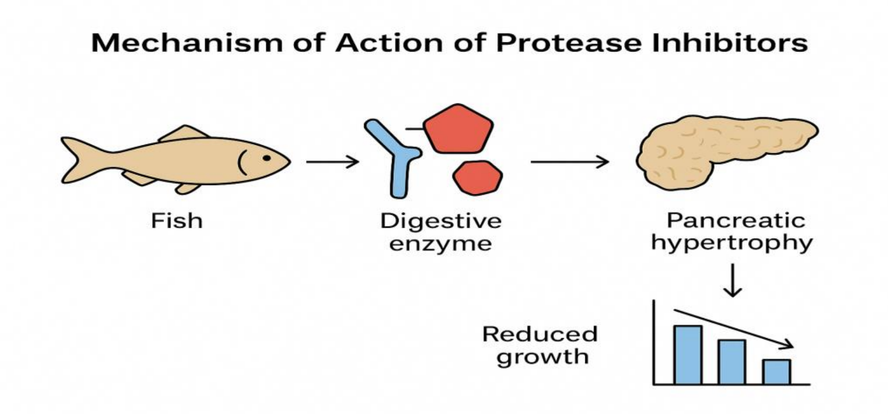
Sources of Protease Inhibitors in Fish Feed

Protease inhibitors (PIs) are widely distributed in plant-derived protein sources commonly used in aquafeeds. Soybean meal is the most significant source, containing both trypsin inhibitors (TI) and chymotrypsin inhibitors (CI), which can account for up to 6–7% of total protein in raw soybeans (Krogdahl et al., 2010). Other legume-based ingredients such as groundnut meal, lupin seed, beans, and peas also contain varying levels of PIs that interfere with protein digestion in fish (Francis et al., 2001). Additionally, processing residues from oilseed cakes and meals may retain residual protease inhibitor activity depending on the extent of heat treatment applied during processing.

Mechanism of Action of Protease Inhibitors

Protease inhibitors exert their anti-nutritional effects primarily by binding to digestive enzymes such as trypsin and chymotrypsin in the gastrointestinal tract of fish. This binding forms stable enzyme–inhibitor complexes, thereby inactivating the enzymes and reducing their ability to hydrolyze dietary proteins (Krogdahl et al., 2010). As a result, the efficiency of protein digestion is compromised, leading to poor amino acid availability for metabolic processes, growth, and tissue development.

To compensate for the loss of active enzymes, the pancreas increases enzyme secretion, which can cause pancreatic hypertrophy and hyperplasia (Francis et al., 2001). While this compensatory mechanism temporarily maintains digestive capacity, it imposes a significant energy cost, diverting resources away from growth and other physiological functions. Chronic exposure to dietary protease inhibitors thus leads to reduced feed conversion efficiency, growth retardation, and nutrient wastage, which may ultimately affect fish health and aquaculture profitability.



Effects on Fish Health and Performance

The presence of protease inhibitors (PIs) in aquafeeds has several detrimental effects on fish health and overall performance. The most direct consequence is growth reduction, caused by poor protein digestibility and decreased amino acid availability. This reduced nutrient utilization translates into nutrient wastage, which is reflected in a higher feed conversion ratio (FCR), lowering feed efficiency and increasing production costs.

Chronic ingestion of PIs may also lead to gut irritation and morphological changes, including shortened microvilli, altered intestinal mucosa, and compromised absorptive capacity. At the same time, continuous over-secretion of digestive enzymes to compensate for enzyme inhibition places the pancreas under heavy stress, leading to pancreatic hypertrophy and hyperplasia.

Nutrient deficiencies caused by impaired protein digestion can also result in indirect immune suppression, as essential amino acids are critical for the synthesis of immune-related proteins and enzymes. Consequently, fish exposed to high PI levels often show reduced resistance to infections and environmental stressors.

Importantly, the severity of these effects is species-dependent. Carnivorous fish such as salmonids and seabass are more sensitive to dietary PIs due to their higher reliance on efficient protein digestion, whereas omnivorous and herbivorous species like tilapia and carp generally show greater tolerance, though still with measurable performance losses at high inclusion levels of plant proteins.

Challenges in Fish Feed Formulation

The incorporation of plant-derived protein ingredients in aquafeeds presents several challenges due to the presence of protease inhibitors (PIs). One major issue is that high inclusion levels of soybean meal, the most common fishmeal replacer, substantially increase the dietary PI burden. This reduces protein digestibility and can significantly impair fish growth performance, particularly in carnivorous species.

Another complication is the variability of PI content across feed ingredients. Factors such as plant variety, cultivation conditions, and processing methods (e.g., oil extraction, heat treatment) strongly influence residual PI levels. This inconsistency makes it difficult to predict the exact impact of PIs on feed performance.

Species differences add to the challenge. Carnivorous fish such as salmonids and seabass are more sensitive to dietary protease inhibitors, while omnivorous and herbivorous species like tilapia and carp show relatively greater tolerance. Feed formulators must therefore account for species-specific nutritional requirements and digestive physiology when determining inclusion levels of plant proteins.

Finally, there is an economic trade-off between cost and nutritional safety. Plant proteins are cheaper and more sustainable than fishmeal, but excessive reliance on them can reduce feed efficiency due to PI-related issues, ultimately negating cost savings. Optimizing feed formulations to balance affordability, nutritional quality, and fish health remains a critical challenge for the aquafeed industry

Strategies for Detoxification of Protease Inhibitors

Effective management of protease inhibitors (PIs) is essential to ensure the nutritional safety of plant-based aquafeeds. Several strategies have been developed to reduce their negative impact on fish growth and health, ranging from feed processing to advanced biotechnological approaches.

Processing Techniques

Thermal processing methods such as toasting, extrusion, and autoclaving are widely used to inactivate protease inhibitors. Heat denatures the inhibitor proteins, thereby reducing their capacity to bind digestive enzymes. However, excessive heating may also destroy essential amino acids, lowering the nutritional value of feed. Other methods such as soaking, germination, and microbial fermentation can also reduce PI levels. Fermentation with beneficial microbes not only decreases inhibitor activity but may also enhance the digestibility and palatability of plant ingredients.

Enzymatic Approaches

The supplementation of aquafeeds with exogenous proteases offers a promising strategy to overcome PI effects. These enzymes help to compensate for inhibited endogenous proteases, improving protein digestion and nutrient utilization. When combined with other feed enzymes such as phytase, synergistic benefits on nutrient availability have been reported.

Biotechnological Strategies

Advances in crop biotechnology have enabled the development of genetically modified soybean and legume varieties with reduced protease inhibitor content. Such innovations provide long-term, sustainable solutions by reducing the need for extensive processing, although acceptance and regulatory approval remain major challenges.

Feed Formulation Approaches

Balanced feed formulation can also help minimize the impact of PIs. Strategies include blending plant proteins with animal proteins such as fishmeal or insect meal to dilute inhibitor concentrations. Additionally, the inclusion of functional feed additives like probiotics, prebiotics, and organic acids can improve gut health and enhance the digestive capacity of fish, indirectly alleviating the adverse effects of protease inhibitors.

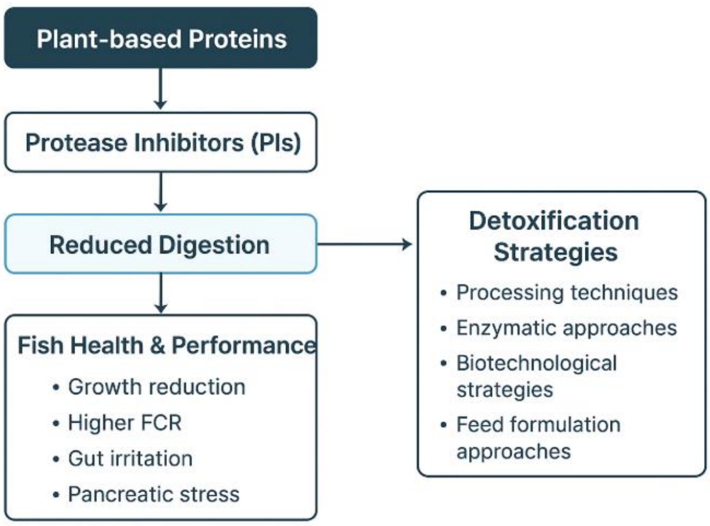
Recent Advances & Case Studies

Recent studies highlight multiple approaches to mitigate protease inhibitors (PIs) in aquafeeds. Enzyme supplementation, particularly with proteases, improves protein digestibility; when combined with phytase, synergistic gains in nutrient utilization and growth are observed (Xue et al., 2024). Microbial fermentation of soybean meal using *Bacillus* or *Aspergillus* can reduce trypsin inhibitor activity by >80%, while also enhancing palatability and amino acid availability (Fermentation MDPI, 2024). Advances in breeding low-KTI **(**Kunitz Trypsin Inhibitor) soybean cultivars offer long-term solutions, though regulatory and adoption challenges remain (USDA/ARS, 2024).

Case studies show species-specific responses: tilapia and carp benefit significantly from protease supplementation or fermented soybean meal; salmonids remain highly sensitive, requiring thorough PI reduction; and catfish/shrimp show improved growth with protease–phytase blends (Krogdahl et al., 2010; Francis et al., 2001). Collectively, combining processing, fermentation, enzyme supplementation, and genetic improvement provides the most effective strategy for controlling PIs in aquafeeds.

Conclusion

Protease inhibitors (PIs) continue to pose a significant challenge in the utilization of plant-based proteins for aquafeeds. Their interference with digestive enzymes reduces protein utilization, compromises fish health, and increases feed costs through poor efficiency. Adoption of effective detoxification techniques—such as heat processing, fermentation, enzyme supplementation, and selective breeding of low-PI crops—offers practical solutions to mitigate these effects. Integrating such strategies into feed formulation is essential not only for improving fish growth and welfare but also for ensuring the long-term sustainability and economic viability of aquaculture



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