



OVERVIEW OF THE USE OF EXOGENOUS ENZYMES IN AQUACULTURE: THE FUNCTIONALITY OF EXOGENOUS ENZYMES IN AQUACULTURE

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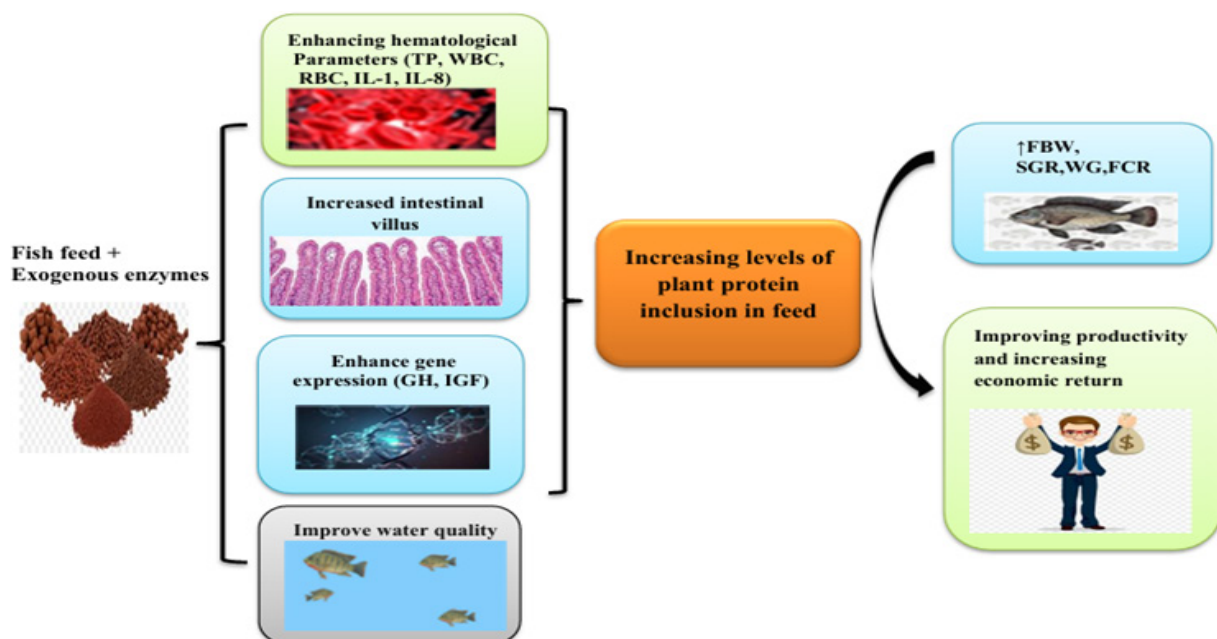
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Abstract

Aquaculture is playing a pivotal role in meeting the growing global request for seafood products. One of the important aspects of aquaculture management is using of high-quality ingredients to improve the performance of fish and crustaceans. One of the main priorities of nutrition scientists over the past decades is to partially or totally replace fish meal (FM) with different plant protein ingredients. Furthermore, plant proteins contain anti-nutritional components, which poses a drawback for their use in fish feed. Therefore, to effectively utilize plant components in feeds, it is necessary to reduce anti-nutritional factors. Recently, exogenous enzymes as feed additives are gaining interest in the aquafeed industry to increase the nutritive values of plant protein as a reasonable solution for FM substitution, leading to enhanced nutrient retention, growth performance and profits for the aquaculture stakeholders. The present review will discuss the potential of various exogenous enzymes to increase the quality of various plant proteins in feed for more sustainable aquaculture practices.

Key words: aquaculture, fish meal, plant protein, fish feed, exogenous enzymes, aquafeed

Graphical abstract



In recent years, the demand for aquatic animal protein has been increasing much faster than the supply of capture fisheries, and the gap has been filled by aquaculture worldwide (Prachom et al., 2023; Mohammady et al., 2024; Ahmed et al., 2025 a). Aquaculture farming totally relies on nutritionally balanced, high-quality aquafeeds (Sherine et al., 2024; Ahmed et al., 2025 b). Therefore, the trend is shifting from fishery-based fish meal to plant-based ingredients to meet the increasing feed demand and ensure sustainable growth (Goda et al., 2019; Jastaniah et al., 2024; Hussain et al., 2024; Anwar et al., 2025). But plant-based ingredients have negative effects on feed because they contain a wide range of anti-nutritional factors (ANFs), which lowers the fish performance (Hassaan et al., 2021; Maas et al., 2021; Sherine et al., 2023). Therefore, to effectively utilize plant components in feeds, it is necessary to reduce anti-nutritional effects (Ragab et al., 2023; Azcuy et al., 2024). Using of plant protein is accompanied by enhancing feed processing technology to break down ANFs (Hassan et al., 2018; Goda et al., 2019). Nowadays, incorporation of exogenous enzymes in fish feeds as functional additives has received great attention to increase the use of plant proteins in aquafeeds (Hassaan et al., 2019 a, b, 2020). This is because it helps degrade large amounts of anti-nutritional factors, thus improving the availability and palatability of nutrients in feeds (Zhang et al., 2023 a, b; Rehemujiang et al., 2023), which increases the incorporation of plant proteins into feed (Goda et al., 2020 a, b; Soltan et al., 2023; Elhalis et al., 2023; Awad et al., 2024). The use of exogenous enzymes in fish feed has led to increased substitution of FM by plant-based ingredients (Huang et al., 2020; Luo et al., 2020; Monier, 2020) by: i) alleviating the undesirable impacts of ANFs (Hassaan et al., 2020; Shekarabi et al., 2022), ii) hydrolyzing the protein into amino acids; iii) increasing nutrients availability (Liu et al., 2018; Hassaan et al., 2018, 2021); iv) enhancing the fish's health and immune response (Anwar

et al., 2020); v) improving water quality; vi) facilitating the possibility of high incorporation levels of plant protein (Hassaan et al., 2019 a, b; Chuchird et al., 2023) and vii) enhancing productivity and increasing economic return (Goda et al., 2020 a, b). Furthermore, aquafeed research has shown the possibility of substituting FM by plant proteins up to 30%, whether specified or combined in the feed, without affecting fish performance, but with the inclusion of exogenous enzymes, the replacement can reach 100% of FM (Sánchez-Alcade et al., 2023). Incorporation of exogenous enzymes in aquafeeds is expected to increase by 5.0% by 2022–2028, standing up as a witness of increasing their role in aquafeed industry (LP Information, Inc., 2022; Sureshkumar et al., 2023). The present review will discuss the roles of functional exogenous enzymes to reduce or eliminate ANFs in ingredients originated from plant sources on performance and the nutritional value of various plant proteins in feed, highlighting future opportunities for sustainable aquaculture practices.

Types of feed enzymes

Among the common exogenous enzymes in aquafeeds, phytase, carbohydrase, and protease are the most commonly used. Figure 1 shows the common exogenous enzymes in aquafeeds, each enzyme has its own biological function and hydrolyzes different ANFs as: i) Phytase catalyzes phytate, and improves the bio-availability of minerals, mainly calcium and phosphorus (Encarnação, 2016; Lemos and Tacon, 2017); ii) Proteases improve protein digestion (Menezes-Blackburn and Greiner, 2015); iii) Carbohydrase digests different carbohydrates (Al Loman and Ju, 2017), and leads to enhance carbohydrates utilization from plant-based ingredients (Wang et al., 2016). Table 1 shows the effects of using exogenous enzymes on growth and physiological processes in various species of fish and crustaceans.

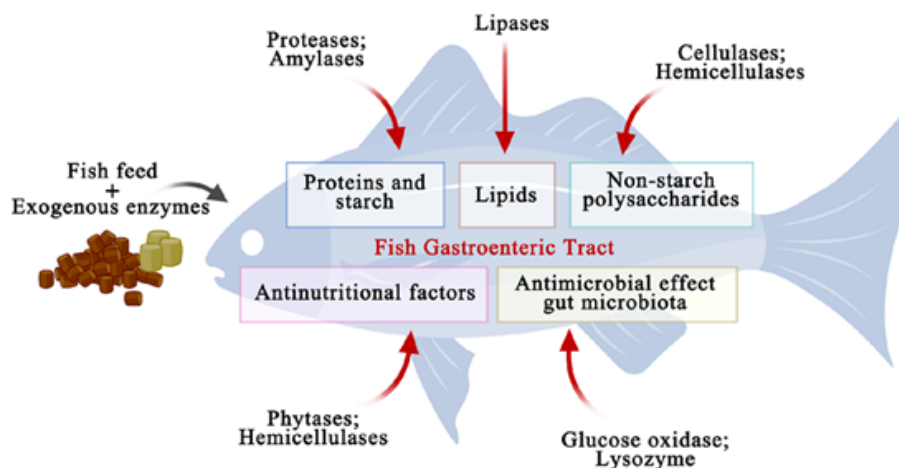


Figure 1. Effects of enzymes on fish nutrition (Liang et al., 2022)

Table 1. Some studies on the effects of different types of exogenous enzymes with plant protein diet on growth and physiological functions in fish and crustaceans

Enzyme type	Fish species	Dose	Effect	Reference
Mixture of enzymes (protease, pepsin, trypsin, xylanase, amylase)	Nile tilapia, <i>Oreochromis niloticus</i>	0.5 g kg ⁻¹ replacement of FM	↑growth; ↑digestibility; ↑digestive enzyme; ↑intestinal morphology; ↑blood indices; ↑immune response	Soltan et al., 2023
Mixture of enzymes (xylanase, amylase, protease)	Rabbitfish, <i>Siganus rivulatus</i>	3 g kg ⁻¹	↑growth performance; ↑feed utilization; ↑health conditions	Dawood et al., 2019
Mixture of enzymes (xylanase, amylase, lipase, protease)	Common carp, <i>Cyprinus carpio</i>	1.0 g/kg	↑growth performance; ↑health of fish	Monier, 2020
Mixture of enzymes (amylase, xylanase, protease, phytase)	Pangas catfish, <i>Pangasius hypophthalmus</i>	3 g kg ⁻¹	↑growth performance; ↑feed utilization; ↑nutritional quality	Bala et al., 2020
Mixture of enzymes (phytase, protease)	Pacific white shrimp, <i>Penaeus vannamei</i>	1 g kg ⁻¹	↑growth performance; ↑feed utilization	Chuchird et al., 2023
Mixture of enzymes (protease, xylanase)	Japanese seabass, <i>Lateolabrax japonicus</i>	1.5–2.0 g/kg	↑growth performance; ↑digestive enzyme activity; ↑non-specific immunity; ↓feed costs	Huang et al., 2020
Xylanase	Nile tilapia, <i>Oreochromis niloticus</i>	0.5 g/kg ⁻¹	↑growth digestive enzymes; ↑nutrient digestibility; ↑histological morphometry of liver and intestine and nutrient retention;	Hassaan et al., 2019
	Nile tilapia, <i>Oreochromis niloticus</i>	up to 0.5 g kg ⁻¹		El-Ashry et al., 2021
	Juvenile mori, <i>Cirrhinus mrigala</i>			Nadeem et al., 2022
Protease	Nile tilapia, <i>Oreochromis niloticus</i>	5.5 g/kg ⁻¹	↑growth performance; ↑feed utilization; ↑intestinal health; ↑immunity; ↑intestinal histological; ↑biochemical blood; ↑metabolic gene expression; ↑intestinal bacterial; ↑digestive enzyme activity; ↑alter gene expression of GH and IGF-I; ↓feed costs	Wu et al., 2020
	Nile tilapia, <i>Oreochromis niloticus</i>	5 g kg ⁻¹		Hassaan et al., 2020
	European sea bass, <i>Dicentrarchus labrax</i>	0.5 g kg ⁻¹		Goda et al., 2020 b
	Nile tilapia, <i>Oreochromis niloticus</i>	2.5 g kg ⁻¹		Hassaan et al., 2019
	Gilt head sea bream, <i>Sparus aurata</i>	0.5 g kg ⁻¹		Özcan and Tasbozan, 2022
	Gibel carp, <i>Carassius auratus gibelio</i>	150–175 mg/kg		Shi et al., 2016
	Nile tilapia, <i>Oreochromis niloticus</i>	2.5 g kg ⁻¹		Saleh et al., 2022
	Nile tilapia, <i>Oreochromis niloticus</i>	0.5 g kg ⁻¹		Hassaan et al., 2021
Phytase	Grass carp, <i>Ctenopharyngodon idella</i>	1.0–0.5 kg ⁻¹		Feng et al., 2023
	Nile tilapia, <i>Oreochromis niloticus</i>	300 mg kg ⁻¹		Adeoye et al., 2016
	European sea bass, <i>Dicentrarchus labrax</i>	0.5 g kg ⁻¹		Goda et al., 2020 a
	Turbot, <i>Psetta maxima</i> L.	2 g kg ⁻¹	↑growth performance; ↑feed efficiency; ↑nutrient bioavailability; ↓feed costs; ↑gene expression; ↑immunity; ↑intestinal histological; ↓feed costs	Danwitz et al., 2016
	Nile tilapia, <i>Oreochromis niloticus</i>	1.5–2 g kg ⁻¹		Rodrigues et al., 2023
	Rohu carp, <i>Labeo rohita</i>	1 g kg ⁻¹		Shah et al., 2021
	Rainbow trout, <i>Oncorhynchus mykiss</i>	2.5 g kg ⁻¹		Azcuy et al., 2024
	Nile tilapia, <i>Oreochromis niloticus</i>	1500 FTU/kg		Negm, et al., 2024
	Red drum, <i>Sciaenops ocellatus</i>	5520 FTU/kg		Chen and Gatlin, 2024
	Common carp, <i>Cyprinus carpio</i>	750 FTU kg ⁻¹		Hussain et al., 2024
Cacarbohydrase enzymes	White seabream, <i>Diplodus sargus</i>	40 mg/kg	↑growth; ↑digestive enzyme activities; ↑carbohydrate metabolic enzymes; ↑ <i>glut2</i> gene expression in the intestine; ↓feed costs	Magalhães et al., 2016
	Pacific white shrimp, <i>Litopenaeus vannamei</i>	100 mg/kg		Wenxiang et al., 2018
	Hybrid grouper, <i>Epinephelus fuscoguttatus</i> × <i>Epinephelus lanceolatus</i>			Liu et al., 2023 b
α-amylase	Olive flounder, <i>Paralichthys olivaceus</i>		↑digestibility; ↑growth-related; ↑gene expression	Hasan et al., 2023

↑:increase;↓:deficiency.

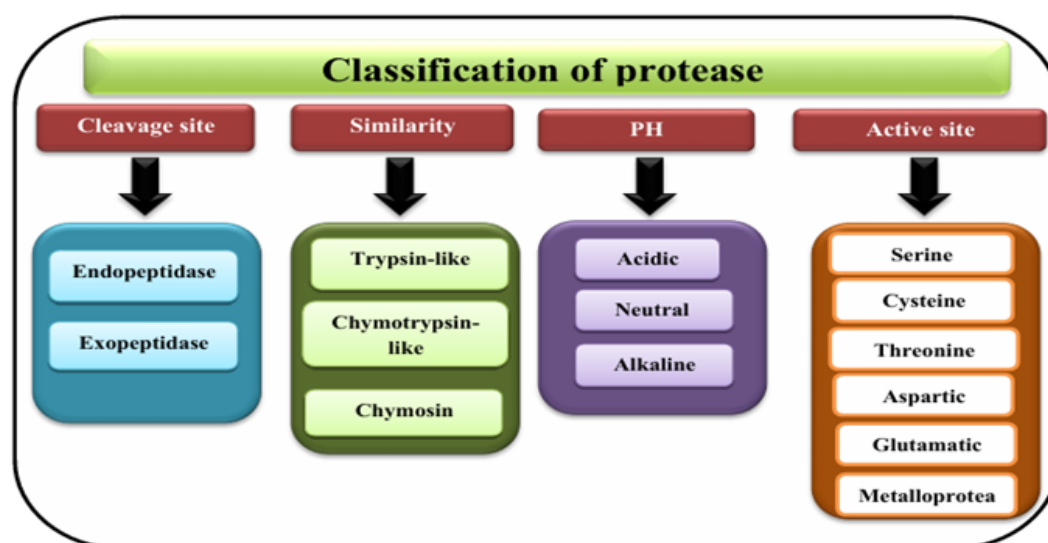


Figure 2. Classification of protease

Proteases

Protease enzymes are known as proteolytic enzymes (Farooq et al., 2024) and are the most common enzymes included in aquafeeds for assimilating proteins, and their use in fish diets has been increasing quickly in aquatic feed in recent years (Goda et al., 2020 b). They are divided into three types: animal, plant and microbial-derived proteases (Islam et al., 2022; Wu et al., 2024; Chen et al., 2024). The protease enzyme used in fish diets is usually extracted from microorganisms as bacteria and fungi (Anadón et al., 2019). However, the common factors that have an effect on enzyme activity are pH and temperature (Awad et al., 2020; Buchholz et al., 2020). Microbial proteases are the most used in the feed industry (Aguilar and Sato, 2018; Chen and Gatlin, 2024 b; Farooq et al., 2024). Proteases are classified into four categories based on: 1) cleavage site 2) active site 3) similarity 4) pH (García-Carreño, 1992; Rawlings et al., 2004; Sriket, 2014; Maryam et al., 2024). Figure 2 shows the classification according to each category.

The mode of action of protease enzymes to improve the quality of plant proteins could be summarized as follows: i) they interrupt the cell content., ii) improve the link between protease enzyme and cell content, resulting in improved protein quality by breaking down ANFs and the release of unavailable nutrients (Shi et al., 2016; Liu et al., 2018; Hassaan et al., 2019 a, b; Goda et al., 2020 a) and iii) incorporation of protease enzyme improves the endogenous protease activity that consequently enhances amino acids utilization. This could be explained by enhancement of nutrient assimilation and the structure of intestinal histology (Lin et al., 2007). Previous research conducted in different fish species and shrimp reported that the inclusion of protease in low fish meal diet boosts the growth and immune response (Gopalraaj et al., 2023; Goda et al., 2020 b; Hassaan et al., 2018, 2020, 2021;

Saleh et al., 2022; Wu et al., 2020; Chowdhury et al., 2018; Gopalraaj et al., 2023). Hassan et al. (2019 a), Goda et al. (2020 a), and Saleh et al. (2022) noted that feeding Nile tilapia diets enriched with protease improves performance versus basal diet without supplementation. Alike, Maryam et al. (2022) found that inclusion of 4.5/kg⁻¹ protease in poultry by-product meal-based diet (PBM) of tilapia achieved growth and feed efficiency matching control diet containing FM. Also, Kemigabo et al. (2019) found that inclusion of ~1.5 g/kg⁻¹ protease in African catfish resulted in significant enhancement of growth.

Phytase

Recently, low-cost plant protein ingredients have been commonly used in fish feeds, but their natural phytate content can lead to deficiencies of micro- and macro-nutrients, thus reducing the absorption rate and reducing the growth performance (Fontes et al., 2019; Samtiya et al., 2020; Chen and Gatlin, 2024). Therefore, diets enriched with phytase are considered as one of promising protocols to mitigate the negative effects of phytate by controlling excreted nutrients such as P and N and unbind P from phytate acid that fish can use directly for optimal growth (Hussain et al., 2021; Maas et al., 2020; Dias and Santigosa, 2023; Rizwanuddin et al., 2023; Liu et al., 2023 a; Chen and Gatlin, 2024). Studies show that phytase supplements improve the phosphorus, trace minerals, amino acids, and energy bioavailability of plant feeds, in addition to reducing environmental impact and phosphorus loads in the aquatic environment (Lemos and Tacon, 2017). In general, phytase enzymes are used in feed formulation in different forms as spray, coated, dry powder and liquid form on finished pellets to improve phosphorus availability at high feed processing temperatures. The recommended dose of phosphorus ranged from 500 to 4000 FTU/kg of diet at 55–60°C and pH range of 2.5–5.5

to function effectively (Sarfraz et al., 2020; Hussain et al., 2024). Previous research was conducted using phytase enzymes in different fish species to improve growth, feed utilization and nutrient availability as Nile tilapia, European sea bass, rainbow trout and carp, *Labeo rohita* (Rodrigues et al., 2023; Negm et al., 2024; Goda et al., 2020 a; Azcuy et al., 2024, Shah et al., 2021). In this context, Goda et al (2020 b) found that plant-based diets enriched with phytases significantly enhance weight gain and feed efficiency in sea bass. Furthermore, Terrey et al. (2023) reported that Atlantic salmon fed diets containing phytase improved performance. On the contrary, the inclusion of phytase in diet of Nile tilapia and rainbow trout did not have any impact on growth and feed efficiency (Hu et al., 2016; Yigit et al., 2018). The variation among the results of different researches could be associated with several factors related to fish physiology, nutrition and environmental conditions (Yigit et al., 2018; Goda et al., 2020 b). The advantages of using phytase include enhanced mineral absorption, energy and AAs utilization (Bowyer et al., 2020; Sharawy et al., 2020), improved gastrointestinal histology (Rachmawati et al., 2017) and boosted micro- and macro-nutrients assimilation (Goda et al., 2020 a; Chen et al., 2024).

Carbohydrase

Non-starch polysaccharides (NSPs) are one of the common ANFs in plant protein ingredients that limit their use in aquafeeds (Hassaan et al., 2019 a; Lee et al., 2020). Furthermore, presence of NSPs reduces nutrients availability in fish feeds (Dawood and Koshio, 2020; Liu et al., 2023 b). In addition, fish lack the enzymes necessary to digest NSPs, which caused viscidness in the digestive tract and negatively affected growth rate (Kuz'Mina, 1996; Leenhouders et al., 2007). Therefore, the main goal of using carbohydrase's enzymes is to break down complex NSPs that fish cannot digest, and reduce NSPs-induced digesta viscosity (Castillo and Gatlin, 2015), thus improving the

gut health (Adeola and Cowieson, 2011). NSPs belong to the group of complex carbohydrates, making up 25–50% of plant components (Nadeem et al., 2022). Enzymes of CHO digestion improve nutrients digestibility and availability and consequently improve fish performance (Kumar and Chakravarty, 2018; Hasan et al., 2023).

Supplementation of exogenous carbohydrates in various fish species and shrimp has been shown to increase growth, feed conversion ratio, health of fish and gut microbiome community of the gastrointestinal tract (Zamini et al., 2014; Jiang et al., 2014; Zhou et al., 2013; Magalhães et al., 2016; Qiu and Davis, 2016; Wenxiang et al., 2018; Engin and Koyuncu, 2023; Liu et al., 2023 b).

Similarly, Ai et al. (2007) found that substitute of FM with plant proteins enriched with carbohydrase enzyme in Japanese seabass diet improve growth and feed utilization, resulting in decreased levels of NSPs. However, several factors affect the incorporation level of exogenous carbohydrase in aquafeeds as the percentage of the plant protein ingredient in the diet; fish species, age and size; type and quantity of enzymes (El-Husseiny et al., 2018; Zheng et al., 2020). Although supplements containing exogenous carbohydrate enzymes show promising results, their availability is limited and requires further research.

Modes of action

The importance of including exogenous enzymes in the rations having high levels of plant proteins could be attributed to the following scenario: i) hydrolyzed components that cannot be dissolved by endogenous enzymes (Liu et al., 2018); ii) breakdown of ANFs and, consequently, hydrolyzing the protein into amino acids (Hassaan et al., 2021); iii) liberate nutrients associated with the cell (Velázquez-De Lucio et al., 2021); iv) increase the availability of some nutrients (Hassaan et al., 2021; Velázquez-De Lucio et al., 2021); v) increase digestive enzymes activity, resulting in better digestion (Liu et al., 2018).

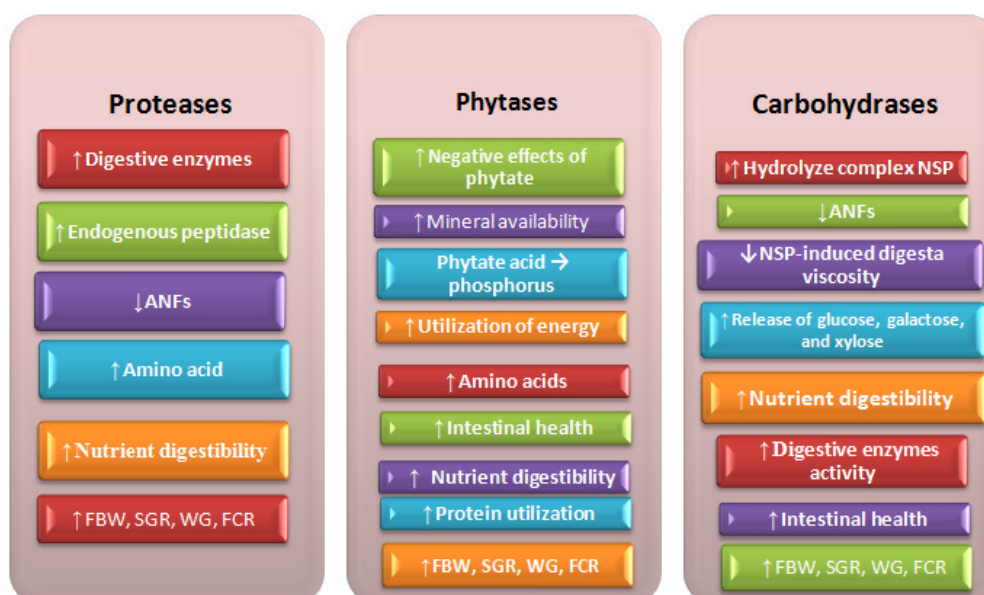


Figure 3. Effects of different exogenous enzymes on improving nutrition value of plant protein and growth

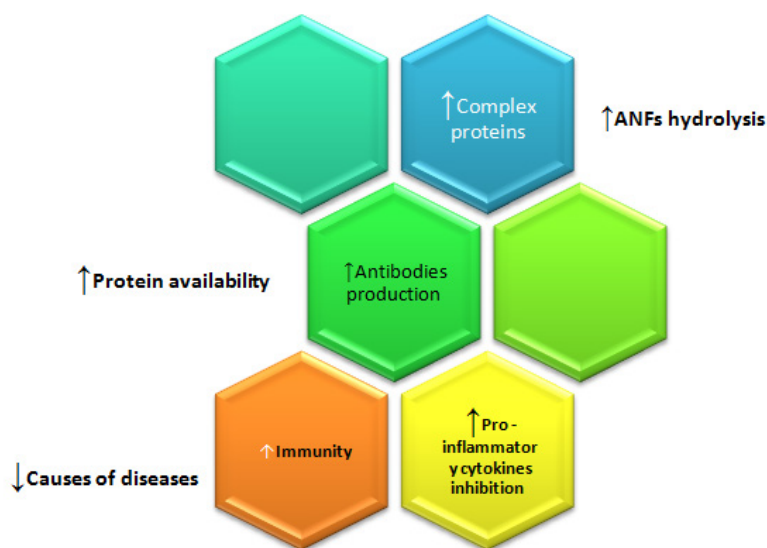


Figure 4. Effects of exogenous enzymes on improving immune response

Impact of exogenous enzymes on various parameters in fish

Recent studies highlight the importance of using exogenous enzymes in the diets of various fish species with high incorporation levels of plant proteins via enhanced performance, improved gut morphology resulting in breakdown of ANFs and enhanced nutrients digestibility and availability (Figure 3; El-Haroun et al., 2009; Hassaan et al., 2020; Soltan et al., 2023; El-Ashry et al., 2021; Wu et al., 2020; Rodrigues et al., 2023; Goda et al., 2020 a, b; Shah et al., 2021; Chuchird et al., 2023).

Growth performance and feed utilization

Growth performance is a sum of several factors including diet chemical composition, types of ingredients and feed additives incorporated (Figure 4; Maryam et al., 2024). Recent studies conducted in various fish species reported that addition of exogenous enzymes in fish fed *plant-based diets* significantly improved growth and digestibility criteria (Shi et al., 2016; Hassaan et al., 2019 b; Li et al., 2016). Following this pattern, Hassaan et al. (2020) found the best growth of Nile tilapia in the group fed plant protein-based diet supplemented with exogenous protease. The present findings could be attributed to the role of exogenous enzyme to enhance nutrients digestion and assimilation that consequently boost and improve fish growth (Hassaan et al., 2021), thus the present review article highlights the importance of including exogenous enzymes in plant-based diet to: i) mitigate to some extent the negative impact of presence of ANFs in plant proteins-based diet, ii) exogenous enzymes digest grain storage proteins and release high amounts of EAAs and energy (Hassaan et al., 2020). Following the same patterns, (Hassaan et al., 2020; Feng et al., 2023; Wu et al., 2024) reported that inclusion of protease enzymes can be used with plant feeds to replace FM in the diet of Nile tilapia and grass carp without negative impact on

growth performance. In addition, Negm et al. (2024) and Azcuy et al. (2024) found that using of phytase enzymes in the diet of tilapia and trout increases growth. Likewise, Hasan et al. (2023) reported that the use of α -amylase enzymes in olive flounder diet had no significant effects on growth. Differences of the enzymes impacts on growth can be attributed to plant protein types and inclusion levels, fish species and age, enzyme types, and inclusion levels in feeds.

Body composition

The body chemical composition reflects the chemical composition of the diet, nutrient digestibility and availability. Maryam et al. (2022) found that inclusion of exogenous enzymes in plant-based diets enhanced the whole-body chemical composition of rohu juveniles versus basal diet. Furthermore (Li et al., 2016; Wu et al., 2024; Negm et al., 2024; Dalsgaard et al., 2012; Azcuy et al., 2024) found that Nile tilapia and rainbow trout recorded significant improvement in body protein retention when fed diets supplemented with phytase. Furthermore, Negm et al. (2024) and Adeshina et al. (2023 a, b) reported that an inclusion of 1.5 g/kg of phytase increased protein and fat content and reduced carbohydrate content, where the phytase enzyme releases nutrients, leading to an increase in the amount of fats and proteins in the body versus basal group (Cowieson et al., 2017; Goda et al., 2020 a).

Nutrient digestibility

The growth of fish is affected by their ability to digest nutrients (Feng et al., 2023). Recently, several studies have reported that inclusion of plant protein supplemented with exogenous enzyme in aquafeeds improved nutrient digestibility in various fish species (Hassaan et al., 2019 a; Huan et al., 2018; Wu et al., 2024; Negm et al., 2024; Liu et al., 2018; Shi et al., 2016; Yao et al., 2019;

Yigit et al., 2018, Azcuy et al., 2024). Following the same patterns, Castillo and Gatlin (2015), Feng et al. (2023) and Wu et al. (2024) stated that enriched plant proteins with exogenous enzymes enhanced nutrient digestibility. Furthermore, Maryam et al. (2022) reported that inclusion of 0.450 g kg⁻¹ protease increased protein digestibility of fish meal-based diet. Also, Liu et al. (2018) reported that adding 1.50 g.kg⁻¹ protease improved crude protein and ether extract versus basal diet. Similarly, Shi et al. (2016), Ragaa et al. (2017) and Hassaan et al. (2019) stated that enrichment of carp and tilapia diets with protease improved nutrient. These results are attributed to its capability of hydrolyzing complex proteins and ANFs (Maryam et al., 2024). On the other hand, Yigit et al. (2018) and Putnuan et al. (2020) found that inclusion of exogenous enzymes in Nile tilapia and rainbow trout had no impact on the CP and EE digestibility. Putnuan et al. (2020) found that the variation of exogenous enzyme inclusion results could be related to fish species, digestion methods, type and dose of enzymes, and processing technique.

Immune response

The immune response of fish can be evaluated through hematological parameters and biochemical indicators (Figure 5; Abd Elnabi et al., 2020; Diab et al., 2023). Inclusion of exogenous enzymes improved immune response resulting in increased protein availability and antibody production in fish (Maryam et al., 2024). Previous research highlights the role of exogenous enzymes to improve immune system response. Maryam et al. (2022) stated a significant increase in biochemical indices of fish fed diets supplemented with protease versus plant-based diet without supplementation. In addition, Goda et al. (2020 a, b) reported that European seabass fed HPDDG (high-protein distiller's dried grains) diets enriched with either protease or phytase had improved biochemical indices. Similarly, Hassaan et al. (2020) found that the highest blood parameters were recorded in Nile tilapia fed diets enriched with exogenous enzymes. Alike, Negm et al. (2024), Adeshina. et al. (2023 a, b) and Zarghi et al. (2022) reported that inclusion of phytase in fish diet improved immune system response, disease resistance and control of disease outbreaks and pathogen infection. The improvements of immune system response were related to the presence of bioactive compounds derived from medicinal plants (Goda et al., 2020 a). In contrast, Adeoye et al. (2016) found no significant effects on hematological parameters of Nile tilapia fed diets containing exogenous enzymes. The discrepancy between results could be related to fish origin, physiological, nutritional and lab conditions (Hasan et al., 2023).

Histology studies

The gastrointestinal tract is a vital organ for digestion and assimilation by stem cells, which act as mediators of the immune response (Hasan et al., 2023). Histological studies extend knowledge on the nutritional

status of fish, as well as the gastrointestinal morphology and the chyme, which is of great importance for nutrient metabolism (He et al., 2020). Plant protein sources contain ANFs that cause inflammation and restructure fish intestine in terms of mucosal fold and disappearance of the supranuclear vacuolization (Anwar et al., 2020). Recent studies reported that inclusion of exogenous enzymes had a positive impact on gut microbial community and enhanced the intestinal health that consequently improved nutrients metabolism (Saleh et al., 2022; Goda et al., 2020 a, b; Abd Elnabi et al., 2020). Huan et al. (2018) found that enriched plant proteins with exogenous enzyme improve assimilation and retention of nutrients, leading to increased intestinal villus length. Similarly, Goda et al. (2020 a, b) found increasing intestinal villi in European seabass fed diets containing exogenous enzymes protease and phytase compared to a plant-based diet without exogenous enzymes. There is a direct relationship between the intestinal villus morphology and the digestibility of crude protein, which leads to improved digestion, absorption, and retention of crude protein and nutrients (Huan et al., 2018). Similarly, Huan et al. (2018) and Hassaan et al. (2021) found that feeds containing plant proteins supplemented with enzymes led to an intestinal morphology of olive flounder and Nile tilapia, which indicates increased nutrients retention. Gut health in fish is controlled by stress generated by ANFs, which negatively affects the intestine morphology, leading to decreased nutrient retention in the gut (Maryam et al., 2024). Negm et al. (2024) stated that addition of enzymes in Nile tilapia diets enhanced the intestine histology in terms of intestinal villi morphology and the number of goblet cells that consequently improve the capability of the intestine to absorb nutrients. Adeshina et al. (2023 a, b) and Liu et al. (2023) found that the use of phytase enzyme (1000 FTU/kg) in the diet of African catfish and Nile tilapia improved intestinal absorption area versus basal diet without supplementation of enzyme, where phytase breaks down phytic acid by dephosphorylation to release inorganic P, releasing minerals for absorption by the intestine, potentially affecting its absorptive capacity (Negm et al., 2024).

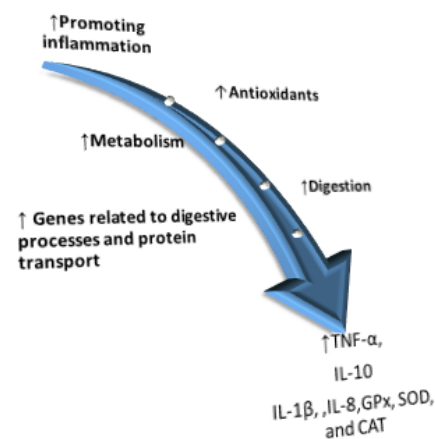


Figure 5. Effects of exogenous enzymes on gene expression

Water quality

Water quality is important to maintaining the health of fish in aquaculture, as water degradation can cause stress and disease outbreaks (Devi et al., 2017; Gopalraaj et al., 2023). Historically, the impact of aquafarms on aquatic ecosystems has been reduced through improvements in feed production, technology, and feed composition (Engin and Koyuncu, 2023). Recently, several studies showed that the use of exogenous enzyme supplementation in fish diets can enhance the digestibility and bioavailability of nitrogen, non-retained phosphorus, and other minerals and significantly reduce the nitrogen and phosphorus load in the aquatic environment, enhancing environmental impact and ensuring sustainable fish production (Goda et al., 2020 a; Moustafa and Amer, 2017). Use of phytase supplementation in plant protein-based diets reduced environmental P load by 30–50% in various fish species (Biswas et al., 2007; Dechavez and Serrano, 2012).

Similarly, Moustafa and Amer (2017) found that adding exogenous enzymes to feeds can reduce aquatic ecosystem pollution by 23% and 8.2% in P and N excretion, respectively. Furthermore, addition of protease in tilapia diet containing lower N and NH_3 levels in their water due to the protease successfully digesting the protein consequently reduced waste outputs in the water (Yuangsoi et al. 2018). Similarly, Baruah et al. (2004) found that feeding fish with high plant protein content leads to low digestibility of phytate-P in fish due to their high consumption of plant protein, which results in most of it being excreted into the water, potentially causing pond pollution.

Antioxidant activity and genes

Antioxidants are components that prevent cells from oxidation by reducing the production of free radicals (Neto et al., 2019). The levels of oxidative enzymes, including SOD, GPX, GPX, and MDA, are good indicators of oxidative status and can act as a biomarker for oxidative stress (Martinez-Alvarez et al., 2005). According to Song et al. (2017) and Wu et al. (2020, 2024), the use of protease supplementation in the diet of carp and shrimp helps to accelerate the activities of oxidative enzymes and total antioxidant capacity, and values decreased MDA, which led to a reduction in the causes of diseases (Uzzau et al., 2002).

Recently, nutritionist have focused in their studies on linking nutrition analysis and genomics using a nutritional genomics approach to enhance the effect of diet components on growth and immune gene expression (Figure 6; Mutch et al., 2005). Recent researches confirmed that fish growth is controlled by GH and IGF axis as well as dietary protein (Company et al., 2001; Pérez-Sánchez et al., 2002; Hassan et al., 2019). Hassan et al. (2019; 2023) and Shi et al. (2017) found that in olive flounder and crucian carp fed diets containing amylase and cellulose, respectively, the levels of GH, TGF- β 3, and IGF-1 were significantly up-regulated in the muscle. Moreover,

exogenous enzymes promote gene expression related to inflammation as tumor necrosis factor- α (TNF- α), interleukin-10 (IL-10), IL-1 β , and IL-8 (Kuebutornye et al., 2020; Hossain et al., 2024; Huo et al., 2019; Maryam et al., 2024). Furthermore, Wu et al. (2020) found that inclusion of 0.5 g/kg protease up-regulates TNF- α and IL-1 β genes expression that consequently enhance disease resistance in tilapia. Moreover, inclusion of protease caused down-regulation of heat shock protein 70 (HSP70) (Wu et al., 2020). Similarly Negm et al. (2024), Adeshina et al. (2023 a, b), Zarghi et al. (2022), Hossain et al. (2024) and Maryam et al. (2024) reported that inclusion of exogenous enzymes promotes expression of IL-1 β , IL-10 and TNF- α in fish.

Economic evaluation

The increasing cost of feed is a significant issue that hinders profitability in fish production (Negm et al., 2024). Therefore, improving the digestibility of feed and nutrients not only enhances fish growth rates but also increases the profitability of aquaculture (Hasan et al., 2023). Negm et al. (2024) and Ali (2021) reported that inclusion of phytase increased the fish yield of Nile tilapia fed diets by 16.5%. Following the same patterns, inclusion of phytase in fish diets increased the yield by 29.5% versus control 2.36% (Negm et al., 2024). Similarly, fish fed diets supplemented with either protease or phytase recorded the best relative feed cost per kg of fish gain (Goda et al., 2020 a, b). Furthermore, Nehad et al. (2019) found inclusion of phytase reduced cost by 41.35%. Moreover, Al-Harhi et al. (2020) found that adding phytase resulted in the maximum economic efficiency. Alike, Khan et al. (2006) found that enzyme supplementation decreased the relative cost of broiler feeds by 4%–18%.

Conclusions and future perspective

Recently, exogenous enzyme additives have successfully improved the growth performance, feed utilization and health status of fish and improved water quality. Therefore, more research should be conducted on estimating exogenous enzyme utilization for different fish species at different water temperatures during different stages of growth. In addition, recently advanced biotechnology has the potential to improve the stability of exogenous enzymes in feed.

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References

- Abd Elnabi H.E., Hassanen G.D.I., Soltan M.A., Dokdok G.A. (2020). Effect of protease and prebiotic mixtures with free fishmeal diets on physiological responses and histological examinations of the red tilapia, *Oreochromis* sp. Egypt. J. Aquat. Biol. Fish., 24: 361–378.

- Adeola O., Cowieson A.J. (2011). Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. *J. Anim. Sci.*, 89: 3189–3218.
- Adeoye A.A., Jaramillo-Torres A., Fox S.W., Merrifield D.L., Davies S.J. (2016). Supplementation of formulated diets for tilapia (*Oreochromis niloticus*) with selected exogenous enzymes: overall performance and effects on intestinal histology and microbiota. *Anim. Feed Sci. Technol.*, <http://dx.doi.org/10.1016/j.anifeedsci.2016.03.002>
- Adeshina I., Akpoilih B.U., Tihamiyu L.O., Badmos A.A., Emikpe B.O., Abdel-Tawwab M. (2023 a). Effects of dietary supplementation with microbial phytase on the growth, bone minerals, antioxidant status, innate immunity and disease resistance of African catfish fed on high soybean meal based diets. *J. Anim. Physiol. Anim. Nutr.*, 107: 733–745.
- Adeshina I., Akpoilih B.U., Udom B.F., Adeniyi O.V., Abdel Tawwab M. (2023 b). Interactive effects of dietary phosphorus and microbial phytase on growth performance, intestinal morphology, and welfare of Nile tilapia (*Oreochromis niloticus*) fed on low fishmeal diets. *Aquaculture*, 563: 738995.
- Aguilar J.G., Sato H.H. (2018). Microbial proteases: production and application in obtaining protein hydrolysates. *Food Res. Int.*, 103: 253–262.
- Ahmed S.R., Van Doan H., Davies S., Hassaan M.S., Goda A.M.A., El-Haroun E. (2025 a). Effect of selenium and nano-selenium applications on biological functions in aquaculture – a review. *Ann. Anim. Sci.*, 25: 905–919.
- Ahmed S.R., Hoseinifar S.H., Van Doan H., Rossi W., Davies S., Goda A.M.A., El-Haroun E. (2025 b). Implication of fermented plant protein ingredients: a critical review of nutrition, physiology and growth: related aspects. *Ann. Anim. Sci.*, DOI: 10.2478/aoas-2025-0010
- Ai Q., Mai K., Zhang W., Xu W., Tan B., Zhang C. (2007). Effects of exogenous enzymes (phytase, non-starch polysaccharide enzyme) in diets on growth, feed utilization, nitrogen and phosphorus excretion of Japanese seabass, *Lateolabrax japonicus*. *Comp. Biochem. Physiol. Part A: Molec. Integr. Physiol.*, 147: 502–508.
- Al Loman A., Ju L.K. (2017). Enzyme-based processing of soybean carbohydrate: Recent developments and future prospects. *Enz. Microb. Technol.*, 106: 35–47.
- Alam M.J., Howlader M.A.R., Pramanik M.A.H., Haque M.A. (2003). Effect of exogenous enzyme in diet on broiler performance. *Int. J. Poult. Sci.*, 2: 168–173.
- Alemayehu T.A., Geremew A., Getahun A. (2018). The role of functional feed additives in Tilapia nutrition. *Fisher Aquacult. J.*, 9: 1g–1g.
- Al-Harthi M.A., Attia Y.A., El-Shafe A.S., Elgandy M.F. (2020). Impact of phytase on improving the utilisation of pelleted broiler diets containing olive by products. *Ital. J. Anim. Sci.*, 19: 310–318.
- Ali S. (2021). Efficacy of supplementation of phytase enzyme to broiler diets with or without di-calcium phosphate on performance and economic appraisal. *Egypt Poult. Sci. J.*, 41: 317–331.
- Anadón A., Ares I., Martínez-Larrañaga M.R., Martínez M.A. (2019). Enzymes in feed and animal health. In: *Nutraceuticals in Veterinary Medicine*, Gupta R.C., Srivastava A., Lall R. (eds). Springer, pp. 303–313.
- Anwar A., Wan A.H., Omar S., El-Haroun E., Davies S.J. (2020). The potential of a solid-state fermentation supplement to augment white lupin (*Lupinus albus*) meal incorporation in diets for farmed common carp (*Cyprinus carpio*). *Aquac. Rep.*, 17: 100348.
- Anwar A., Omar S., Mohammed S., El-Haroun E., Ragab S., Van Doan H. (2025). Common Carp (*Cyprinus carpio*) exposure to water-soluble fraction of crude oil (WSF): Evaluation of protective effects of dietary *Rosmarinus officinalis* leaf on water quality, growth performance, oxidative stress, stress hormones and heat shock proteins. *Ann. Anim. Sci.*, 25: 1035–1044.
- Awad A., Mohammady E.Y., Souady M.R., Rabetimarghezar N., El-Haroun E.R., Hassaan M.S. (2024). Growth and physiological response of Nile tilapia (*Oreochromis niloticus*) fed a fermented mixture of plant protein sources. *Anim. Feed Sci. Technol.*, 315: 116034.
- Awad G.E.A., Ghanem A.F., Abdel Wahab W.A., Wahba M.I. (2020). Functionalized κ -carrageenan/hyperbranched poly(amidoamine) for protease immobilization: thermodynamics and stability studies. *Int. J. Biol. Macromol.*, 148: 1140e55.
- Azcuy R.L., Casaretto M.E., Márquez L., Hernández A.J., Morales G.A. (2024). Evaluation of phytase impact on *in vitro* protein and phosphorus bioaccessibility of two lupin species for rainbow trout (*Oncorhynchus mykiss*). *Aquacult. Nutr.*, 2697729.
- Bala P., Mustafa M.d.G., Mandal S.C. (2020). Improved growth and nutritional quality of pangas catfish, *Pangasius hypophthalmus* (Sauvage, 1878) by enzyme supplementation. *Aquacult. Stud.*, 20: 133–142.
- Baruah K., Sahu N.P., Pal A.K., Debnath D. (2004). Dietary phytase: an ideal approach for a cost effective and low polluting aqua feed. *NAGA World. Fish. Centre Quarterly*, 27: 15–19.
- Biswas A.K., Kaku H., Ji S.C., Seoka M., Takii K. (2007). Use of soybean meal and phytase for partial replacement of fish meal in the diet of red sea bream, *Pagrus major*. *Aquaculture*, 267: 284–291.
- Bowyer P., El-Haroun E.R., Davies S.J. (2020). Benefits of a commercial solid-state fermentation (SSF) product on growth performance, feed efficiency and gut morphology of juvenile Nile tilapia (*Oreochromis niloticus*) fed different UK lupin meal cultivars. *Aquaculture*, 523: 735192.
- Buchholz I., Nagel F., Klein A., Wagh P.R., Mahajan U.M., Greinacher A., Lerch M.M., Mayerle J., Delcea M. (2020). The impact of physiological stress conditions on protein structure and trypsin inhibition of serine protease inhibitor Kazal type 1 (SPINK1) and its N34S variant. *Biochim. Biophys. Acta Proteins. Proteom.*, 1868: 140281.
- Castillo S., Gatlin D.M. (2015). Dietary supplementation of exogenous carbohydrase enzymes in fish nutrition: A review. *Aquaculture*, 435: 286–292.
- Chen K., Gatlin D.M. (2024). Optimal dietary supplementation of phytase for red drum (*Sciaenops ocellatus*) based on growth and mineral utilization. *Aquaculture*, 581: 740383.
- Chen S., Maulu S., Wang J., Xie X., Liang X., Wang H., Wang J., Xue M. (2024). The application of protease in aquaculture: Prospects for enhancing the aquafeed industry: Review Article. *Anim. Nutr.*, 16: 105–121.
- Chowdhury M.A.K., Zhu J., Cai C., Ye Y., He J. (2018). Dietary protease modulates nutrient retention efficiency and hepatopancreatic protease activity in juvenile Chinese mitten crab *Eriocheir sinensis*. *Aquacult. Nutr.*, 24: 911–917.
- Chuchird N., Rairat T., Keetanon A., Seguin D., Chotikachinda R., Manomaitis L., Kanjanamayoon C. (2023). Effect of feed enzymes and functional immunostimulants supplementation on growth performance and overall health of postlarvae and juvenile Pacific white shrimp, *Penaeus vannamei*, fed soybean-based diets. *J. World Aquacult. Soc.*, 12939: 1–14.
- Company R., Astola A., Pendón C., Valdivia M.M., Pérez-Sánchez J. (2001). Somatotrophic regulation of fish growth and adiposity: Growth hormone (GH) and somatolactin (SL) relationship. *Comp. Biochem. Physiol.*, 130: 435–445.
- Cowieson A.J., Ruckebusch J.P., Sorbara J.O.B., Wilson J.W., Guggenbuhl P., Roos F.F. (2017). A systematic view on the effect of phytase on ileal amino acid digestibility in broilers. *Anim. Feed Sci. Technol.*, 225: 182–194.
- Dalsgaard J., Verlhac V., Hjermslev N.H., Ekmann K.S., Fischer M., Klausen M., Pedersen P.B. (2012). Effects of exogenous enzymes on apparent nutrient digestibility in rainbow trout (*Oncorhynchus mykiss*) fed diets with high inclusion of plant-based protein. *Anim. Feed Sci. Technol.*, 171: 181–191.
- Danwitz A., van Bussel C.G.J., Klatt S.F., Schulz C. (2016). Dietary phytase supplementation in rapeseed protein based diets influences growth performance, digestibility and nutrient utilization in turbot (*Psetta maxima* L.). *Aquaculture*, 450: 405–411.
- Dawood M.A.O., Koshio S. (2020). Application of fermentation strategy in aquafeed for sustainable aquaculture. *Rev. Aquacult.*, 12: 987–1002.
- Dawood M., Mohsen M., El-dakar A., Abdelraouf E., Moustafa E., Ahmed H. (2019). Effectiveness of exogenous digestive enzymes

- supplementation on the performance of rabbitfish (*Siganus rivulatus*). Slov. Vet. Res., 56: 409–419.
- Dechavez R.B., Serrano A.E. Jr (2012). Evaluation of phytases of three *Bacillus* spp. in the diet of sex-reversed *Oreochromis mossambicus* fingerlings on growth, feed efficiency and mineral deposition. Ann. Biol. Res., 3: 4584–4592.
- Devi P.A., Padmavathy P., Aanand S., Aruljothi K. (2017). Review on water quality parameters in freshwater cage fish culture. Int. J. Appl. Res., 3: 114–120.
- Diab A.M., Eldeghaidy E.E., Abo-Raya M.H., Shukry M., Abdeen A., Ibrahim S.F., Fericean L., Abdo M., Khalafalla M.M. (2023). Assessment of growth-related parameters, immune-biochemical profile, and expression of selected genes of red tilapia fed with roselle calyces (*Hibiscus sabdariffa*) extract. Fishes, 8: 172.
- Dias J., Santigosa E. (2023). Maximizing performance and phosphorus utilization of warm water fish through phytase supplementation. Aquaculture, 569.
- El-Ashry M.A., Radwan A.A., El-Sayed A.I.M., Soltan M.A., Mehrim A.I. (2021). Effect of dietary xylanase on growth performance, digestive enzymes and physiological responses of Nile tilapia, *Oreochromis niloticus* fingerlings fed plant-based diets. Anim. Biotechnol., 59: 71–80.
- Elhalis H., See X.Y., Osen R., Chin X.H., Chow Y. (2023). Significance of fermentation in plant-based meat analogs: a critical review of nutrition, and safety-related aspects. Foods, 12: 3222.
- El-Haroun E.R., Azevedo P.A., Bureau D.P. (2009). High dietary incorporation levels of rendered animal protein ingredients on performance of rainbow trout *Oncorhynchus mykiss* (Walbaum, 1972). Aquaculture, 290: 269–274.
- El-Husseiny O.M., Hassana M.I., El-Haroun E.R., Suloma A. (2018). Utilization of poultry by-product meal supplemented with L-lysine as fish meal replacer in the diet of African catfish *Clarias gariepinus* (Burchell, 1822). J. Appl. Aquac., 30: 63–75.
- Encarnação P. (2016). Functional feed additives in aquaculture feeds. In: Aquafeed formulation, Nates S.F. (ed.). Academic Press, pp. 217–237.
- Engin K., Koyuncu C.E. (2023). The recent advances to increase nutrient utilization of dietary plant proteins by enzyme supplementation and fermentation in rainbow trout (*Oncorhynchus mykiss*): a review. J. Agricult. Sci. (Tarim Bilimleri Dergisi), DOI: 10.15832/ankutbd.1192888
- Farooq K., Anwar Z., Khalid W., Hasan S., Afzal F., Zafar M., Ali U., Alghamdi O., Al-Farga A., Al-Maaqar S.M. (2024). Optimization and detergent compatibility of protease produced from *Aspergillus oryzae* by utilizing agro wastes. ACS Omega, 9.
- Feng L., Feng L., Jiang W.D., Liu Y., Zhang L., Kuang S.Y., Ren H.M., Jin X.W., Li S.W., Mi H.F., Zhou X.Q., Wu P. (2023). The beneficial effects of exogenous protease K originated from *Parengyodontium album* on growth performance of grass carp (*Ctenopharyngodon idella*) in relation to the enhanced intestinal digestion and absorption capacities. Aquaculture, 563: 738929.
- Fontes T.V., Batista de Oliveira K.R., Almeida I.L.G., Orlando T.M., Rodrigues P.B., Vicente da Costa D., Vieira R.P. (2019). Digestibility of insect meals for Nile tilapia fingerlings. Animals, 9: 181.
- Goda A.A.S., Srour T.M., Omar E., Mansour A.T., Baromh M.Z., Mohamed S.A., Davies S.J., El-Haroun E.R. (2019). Appraisal of a high protein distiller's dried grain (DDG) in diets for European sea bass, *Dicentrarchus labrax* fingerlings on growth performance, haematological status and related gut histology. Aquacult. Nutr., 25: 808–816.
- Goda A.M.A.S., Sherine R.A., Nazmi H.M., Ahmad M.A., Mostafa K.S.T., Susan H.F., Baromh Z.M., El-Haroun E.R., Simon D. (2020 a). Assessment of a high protein distillers dried grain (HP-DDG) augmented with phytase in diets for European sea bass, *Dicentrarchus labrax* fingerlings on growth performance, haematological status, immune response and related gut and liver histology. Aquaculture, 529: 735617.
- Goda A.M.A.S., Ahmed S.R., Nazmi H.M., Baromh M.Z., Kevin F., Waldemar R.J., Simon D., El-Haroun E.R. (2020 b). Partial replacement of dietary soybean meal by high-protein distiller's dried grains (HPDDG) supplemented with protease enzyme for European sea bass, *Dicentrarchus labrax* fingerlings. Aquacult. Nutr., 26: 842–852.
- Gopalraaj J., Velayudhannair K., Arockiasamy J.P., Radhakrishnan D.K. (2023). The effect of dietary supplementation of proteases on growth, digestive enzymes, oxidative stress, and intestinal morphology in fishes – A review. Aquacult. Int., <https://doi.org/10.1007/s10499-023-01191-8>
- Hasan M.d.T., Kim H.J., Hur S.W., Jeong S.M., Kim K.W., Lee S. (2023). Dietary exogenous α -amylase modulates the nutrient digestibility, digestive enzyme activity, growth-related gene expression, and diet degradation rate of olive flounder (*Paralichthys olivaceus*). J. Microb. Biotechnol., 33: 1390–1401.
- Hassaan M., Davies S., Soltan M.A., Mohammady E.Y., Elashry M.A., El-Haroun E.R. (2018). Growth and physiological responses of Nile tilapia, *Oreochromis niloticus* to dietary fermented sunflower meal with *Saccharomyces cerevisiae* and *Bacillus subtilis*. Aquaculture, 495: 592–601.
- Hassaan M.S., El-Sayed A.I.M., Soltan M.A., Iraqi M.M., Goda A.M., Davies S., El-Haroun E.R., Ramadan H.A. (2019). Partial dietary fish meal replacement with cotton seed meal and supplementation with exogenous protease alters growth, feed performance, hematological indices and associated gene expression markers (GH, IGF-I) for Nile tilapia, *Oreochromis niloticus*. Aquaculture, 503: 282–292.
- Hassaan M.S., Mohammady E.Y., Adnan A.M., Abd Elnabi H.E., Ayman M.F., Soltan M.A., El-Haroun E.R. (2020). Effect of dietary protease at different levels of malic acid on growth, digestive enzymes and haemato-immunological responses of Nile tilapia, fed fish meal free diets. Aquaculture, 522: 735124.
- Hassaan M.S., Mohammady E.Y., Soaudy M.R., Elashry M.A., Moustafa M.M., Wassel M.A., El-Haroun E.R., Elsaied H.E. (2021). Synergistic effects of *Bacillus pumilus* and exogenous protease on Nile tilapia (*Oreochromis niloticus*) growth, gut microbes, immune response and gene expression fed plant protein diet. Anim. Feed Sci. Technol., 275P: 114892.
- He M., Li X., Poolsawat L., Guo Z., Yao W., Zhang C. (2020). Effects of fish meal replaced by fermented soybean meal on growth performance, intestinal histology and microbiota of largemouth bass (*Micropterus salmoides*). Aquacult. Nutr., 26: 1058–71.
- Hossain M.K.N., Ishak S.D., Iehata S., Noordiyana M.N., Kader M.A., Abol-Munafi A.B. (2024). Growth performance, fatty acid profile, gut, and muscle histo-morphology of Malaysian mahseer, *Tor tambroides* post larvae fed short-term host associated probiotics. Aquacult. Fish., 9: 35–45.
- Hu J., Ran C., He S., Cao Y., Yao B., Ye Y., Zhang X., Zhou Z. (2016). Dietary microbial phytase exerts mixed effects on the gut health of tilapia: a possible reason for the null effect on growth promotion. Brit. J. Nutr., 115: 1958–1966.
- Huan D., Li X., Chowdhury M.A.K., Yang H., Liang G., Leng X. (2018). Organic acid salts, protease and their combination in fish meal-free diets improved growth, nutrient retention and digestibility of tilapia (*Oreochromis niloticus* $\times$ *O. aureus*). Aquacult. Nutr., 24: 1813–1821.
- Huang Z., Li Z., Xu A., Zheng D., Ye Y., Wang Z. (2020). Effects of exogenous multienzyme complex supplementation in diets on growth performance, digestive enzyme activity and non-specific immunity of the Japanese seabass, *Lateolabrax japonicus*. Aquacult. Nutr., 26: 306–315.
- Huo H.J., Chen S.N., Li L., Nie P. (2019). Functional characterization of IL-10 and its receptor subunits in a perciform fish, the mandarin fish, *Siniperca chuatsi*. Dev. Comp. Immunol., 97: 64–75.
- Hussain S.M., Hanif S., Sharif A., Bashir F., Iqbal H.M.N. (2021). Unrevealing the sources and catalytic functions of phytase with multipurpose characteristics. Catalysis Lett., 152: 1358–1371.
- Hussain S.M., Arsalan M.Z.H., Ahmad N., Ahmad B., Tabassum S., Ali B. (2024). Dietary supplementation of citric acid and phytase in plant-based diets improves mineral bioavailability in common carp, *Cyprinus carpio*. Pakistan. J. Zool., 1–9.
- Islam M., Huang Y., Islam S., Fan B., Tong L., Wang F. (2022). Influence of the degree of hydrolysis on functional properties and antioxidant activity of enzymatic soybean protein hydrolysates. Molecules, 27: 6110.

- Jastaniah S.D., Mansour A.A., Al-Tarawni A.H., El-Haroun E., Munir M.B., Sultan Ayesh M. Saghir, Kari Z.A., Tellez-Isaías G., Bottje W.G., AL-Farga A., Eissa E.H. (2024). The effects of nano-curcumin on growth performance, feed utilization, blood biochemistry, disease resistance, and gene expression in European seabass (*Dicentrarchus labrax*) fingerlings. *Aquacult. Rep.*, 36: 102034.
- Jiang T.T., Feng L., Liu Y., Jiang W.D., Jiang J., Li S.H., Zhou X.Q. (2014). Effects of exogenous xylanase supplementation in plant protein-enriched diets on growth performance, intestinal enzyme activities and microflora of juvenile Jian carp (*Cyprinus carpio* var. *Jian*). *Aquac. Nutr.*, 20: 632–645.
- Kemigabo C., Jere L.W., Sikawa D., Masembe C., Kang'ombe J., Abdel-Tawwab M. (2019). Growth response of African catfish, *Clarias gariepinus* (B.), larvae and fingerlings fed protease-incorporated diets. *J. Appl. Ichthyol.*, 35: 480–487.
- Khan S.H., Sardar R., Siddique B. (2006). Influence of enzymes on performance of broilers fed sunflower-corn based diets. *Pakist. Veterin. J.*, 26: 109–114.
- Kuebutornye F.K.A., Tang J., Cai J., Wang Y.Z., Abarike E.D., Lu Y., Li Y., Afriyie G. (2020). *In vivo* assessment of the probiotic potentials of three host-associated *Bacillus* species on growth performance, health status and disease resistance of *Oreochromis niloticus* against *Streptococcus agalactiae*. *Aquaculture*, <https://doi.org/10.1016/j.aquaculture.2020.735440>
- Kumar S., Chakravarty S. (2018). Amylases. In: *Enzymes in Human and Animal Nutrition*, Nunes C.S., Kumar V. (eds). Elsevier, Amsterdam, The Netherlands, pp. 163–180.
- Kuz'Mina V.V. (1996). Influence of age on digestive enzyme activity in some freshwater teleosts. *Aquaculture*, 10: 25–37.
- Lee S.A., Lupatsch I., Gomes G.A., Bedford M.R. (2020). An advanced *Escherichia coli* phytase improves performance and retention of phosphorus and nitrogen in rainbow trout (*Oncorhynchus mykiss*) fed low phosphorus plant-based diets, at 11 °C and 15 °C. *Aquaculture*, 516: 734549.
- Leenhouders J.I., Ortega R.C., Verreth J.A.J., Schrama J.W. (2007). Digesta characteristics in relation to nutrient digestibility and mineral absorption in Nile tilapia (*Oreochromis niloticus* L.) fed cereal grains of increasing viscosity. *Aquaculture*, 273: 556–565.
- Lemos D., Tacon A.G.J. (2017). Use of phytases in fish and shrimp feeds: a review. *Rev. Aquac.*, 9: 266–282.
- Li X.Q., Chai X.Q., Liu D.Y., Chowdhury M.A.K., Leng X.J. (2016). Effects of temperature and feed processing on protease activity and dietary protease on growths of white shrimp, *Litopenaeus vannamei*, and tilapia, *Oreochromis niloticus* × *O. aureus*. *Aquacult. Nutr.*, 22: 1283–1292.
- Liang Q., Yuan M., Xu L., Lio E., Zhang F., Mou H., Secundo F. (2022). Application of enzymes as a feed additive in aquaculture. *Marine Life Sci. Technol.*, 4: 208–221.
- Lin S., Mai K., Tan B. (2007). Effects of exogenous enzyme supplementation in diets on growth and feed utilization in tilapia, *Oreochromis niloticus* × *O. aureus*. *Aquac. Res.*, 38: 1645–1653.
- Liu F., Li J., Ni H., Azad M.A.K., Mo K., Yin Y. (2023 a). The effects of phytase and non-starch polysaccharide hydrolyzing enzymes on trace element deposition, intestinal morphology, and cecal microbiota of growing–finishing pigs. *Animals: An Open Access Journal from MDPI*, 13: 549.
- Liu H., Pan L., Shen J., Tan B., Dong X., Yang Q., Chi S., Zhang S. (2023 b). Effects of carbohydrase supplementation on growth performance, intestinal digestive enzymes and flora, glucose metabolism enzymes, and *glut2* gene expression of hybrid grouper (*Epinephelus fuscoguttatus* × *E. lanceolatus*) fed different CHO/L ratio diets. *Metabolites*, 13: 98.
- Liu W., Wu J.P., Li Z., Duan Z.Y., Wen H. (2018). Effects of dietary coated protease on growth performance, feed utilization, nutrient apparent digestibility, intestinal and hepatopancreas structure in juvenile gibel carp (*Carassius auratus gibelio*). *Aquacult. Nutr.*, 24: 47–55.
- LP Information Inc. (2022). Global animal feed enzymes market growth 2022–2028. Market Research.com. [https:// www.marketresearch.com/LP-Information-Inc- v4134/ Global-Animal-Feed-Enzymes-Growth-30499852/](https://www.marketresearch.com/LP-Information-Inc- v4134/ Global-Animal-Feed-Enzymes-Growth-30499852/). Accessed Jan 2022.
- Luo J., Li Y., Jin M., Zhu T., Li C., Zhou Q. (2020). Effects of dietary exogenous xylanase supplementation on growth performance, intestinal health, and carbohydrate metabolism of juvenile large yellow croaker, *Larimichthys crocea*. *Fish. Physiol. Biochem.*, 46: 1093–1110.
- Maas R.M., Verdegem M.C.J., Stevens T.L., Schrama J.W. (2020). Effect of exogenous enzymes (phytase and xylanase) supplementation on nutrient digestibility and growth performance of Nile tilapia (*Oreochromis niloticus*) fed different quality diets. *Aquaculture*, 529: 735723.
- Maas R.M., Verdegem M.C.J., Lee C.N., Schrama J.W. (2021). Effects and interactions between phytase, xylanase and β -glucanase on growth performance and nutrient digestibility in Nile tilapia. *Anim. Feed Sci. Technol.*, 271: 114767.
- Magalhães R., Lopes T., Martins N., Díaz-Rosales P., Couto A., Pousão-Ferreira P., Oliva-Teles A., Peres H. (2016). Carbohydrases supplementation increased nutrient utilization in white seabream (*Diplodus sargus*) juveniles fed high soybean meal diets. *Aquaculture*, doi: 10.1016/j.aquaculture.2016.05.019
- Martínez-Álvarez R., Morales A., Sanz A. (2005). Antioxidant defenses in fish: biotic and abiotic factors. *Rev. Fish. Biol. Fish.*, <https://doi.org/10.1007/s11160-005-7846-4>.
- Maryam, Shah S.Z.H., Fatima M., Hussain S.M., Nadeem H., Hussain M. (2022). The effectiveness of protease supplemented poultry by-product meal-based diet on growth, nutrient digestibility and digestive enzyme activities of rohu (*Labeo rohita*). *Aquacult. Res.*, 53: 3841–3852.
- Maryam, Shah S.Z.H., Fatima M., Nadeem H., Ashraf S., Hussain M. (2024). Roles of dietary supplementation of exogenous protease in low fishmeal aquafeed – a mini review. *Ann. Anim. Sci.*, 24: 27–39.
- Menezes-Blackburn D., Greiner R. (2015). Enzymes used in animal feed: Leading technologies and forthcoming developments. In: *Functional Polymers in Food Science*, Cirillo G., Spizzirri U.G., Lemma F. (eds). Scrivener Publishing, pp. 47–73.
- Mohammady E.Y., Elashry M.A., Ibrahim M.S., Elarian M., Salem S.M.R., El Haroun E.R., Hassaan M.S. (2024). Nano iron versus bulk iron forms as functional feed additives: growth, body indices, hematological assay, plasma metabolites, immune, anti oxidative ability, and intestinal morphometric measurements of Nile tilapia, *Oreochromis niloticus*. *Biol. Trace Elem. Res.*, 202: 787–799.
- Monier M.N. (2020). Efficacy of dietary exogenous enzyme supplementation on growth performance, antioxidant activity, and digestive enzymes of common carp (*Cyprinus carpio*) fry. *Fish Physiol. Biochem.*, 46: 713–723.
- Moustafa Y.T.A., Amer T.N.A. (2017). Toward more environmentally friendly aquaculture: effect of dietary exogenous enzymes (Natuzyne®) on nitrogen and phosphorus retention and excretion in Nile tilapia (*Oreochromis niloticus*). *Proc. 1st International Conference (Central Laboratory for Aquaculture Research in Cooperation with Worldfish)*, 2: 478–501.
- Mutch D.M., Wahl W., Williamson G. (2005). Nutrigenomics and nutrigenetics: The emerging faces of nutrition. *FASEB J.*, 19: 1602–1616.
- Nadeem H., Shah S.Z.H., Fatima M., Maryam, Shahzad M.M. (2022). Effects of exogenous xylanase supplementation in plant-based diets on growth performance, nutrient digestibility and digestive enzyme activities of juvenile mori (*Cirrhinus mrigala*). *Anim. Feed Sci. Technol.*, 291: 115391.
- Negm A.E., Abo-Raya M.H., Gabr A.M., Baloza S.H., El-Nokrashy A., Prince A., Arana D., Wan Y., Abdelazeem S., Albadrani G.M., Al-Ghadi M.Q., Abdeen A., Shukry M., El-Sayed Khallafallah M.M. (2024). Effects of phytase enzyme supplementation on growth performance, intestinal morphology and metabolism in Nile tilapia (*Oreochromis niloticus*). *J. Anim. Physiol. Anim. Nutr.*, 13939: 1–18.
- Nehad M., Eid A.E., Ali B.A., Ali W., Enany M.E., Abd El-Naby A.S. (2019). Effect of phytase and citric acid on growth performance, feed utilization and its antibacterial activity against fish pathogens of Nile tilapia fingerlings. *Egypt J. Aquacult.*, 9: 35–53.
- Neto Y.A.H., Rosa J.C., Cabral H. (2019). Peptides with antioxidant properties identified from casein, whey, and egg albumin hydro-

- lysates generated by two novel fungal proteases. *Prep. Biochem. Biotechnol.*, 49: 639–648.
- Özcan F., Tasbozan O. (2022). The effects of dietary protease enzyme on growth performances and digestive enzyme activities of gilt head sea bream (*Sparus aurata*). *Anim. Nutr. Feed Technol.*, 22: 95–106.
- Pérez-Sánchez J., Caldach-Giner J.A., Mingarro M., de Celis S.V.R., Gómez-Requeni P., Saera-Vila A., Valdivia M.M. (2002). Overview of fish growth hormone family. New insights in genomic organization and heterogeneity of growth hormone receptors. *Fish Physiol. Biochem.*, 27: 243–258.
- Prachom N., Yuangsoi B., Pumnuan J., Ashour M., Davies S.J., El-Haroun E. (2023). Effects of substituting the two-spotted cricket (*Gryllus bimaculatus*) meal for fish meal on growth performances and digestibility of striped snakehead (*Channa striata*) Juveniles. *Life*, 13: 594.
- Putnau P., Jintasatoporn Srinoy O.C. (2020). *In vitro* digestibility of fishmeal reduction diet in combination with protease enzyme by Nile tilapia (*Oreochromis niloticus*) digestive enzyme. *Msu Editorial Board for Proceeding*, 44: 1–7.
- Qiu X., Davis D.A. (2017). Effects of dietary carbohydrase supplementation on performance and apparent digestibility coefficients in Pacific white shrimp, *Litopenaeus vannamei*. *J. World Aquac. Soc.*, 48: 313–319.
- Rachmawati D., Istiyanto S., Maizirwan M. (2017). Effect of phytase on growth performance, diet utilization efficiency and nutrient digestibility in fingerlings of *Chanos chanos* (Forsskal 1775). *Philippine J. Sci.*, 146: 237–245.
- Ragaa N.M., Abu Elala N.M., Kamal A.M., Kamel N.F. (2017). Effect of a serine-protease on performance parameters and protein digestibility of cultured *Oreochromis niloticus* fed diets with different protein levels. *Pak. J. Nutr.*, 16: 148–154.
- Ragab S., Hoseinifar S.H., Van Doan H., El-Haroun E. (2023). Evaluation of distiller's dried grains with solubles in aquafeeds – a review. *Ann. Anim. Sci.*, 24: 65–75.
- Rehemjiang H., Yusuf H.A., Ma T., Diao Q., Kong L., Kang L., Tu Y. (2023). Fermented cottonseed and rapeseed meals outperform soybean meal in improving performance, rumen fermentation, and bacterial composition in Hu sheep. *Front. Microbiol.*, 14: 1119887.
- Rizwanuddin S., Kumar V., Naik B. (2023). Microbial phytase: their sources, production, and role in the enhancement of nutritional aspects of food and feed additives. *J. Agricult. Food Res.*, 12: 100559.
- Rodrigues E.J.D., Ito P.I., Ribeiro L.F.M., de Carvalho P.L.P.F., Xavier W.d.S., Guimarães M.G., Junior A.C.F., Pezzato L.E., Barros M.M. (2023). Phytase supplementation under commercially intensive rearing conditions: impacts on Nile tilapia growth performance and nutrient digestibility. *Animals*, 13: 136.
- Saleh E.S.E., Tawfeek S.S., Abdel-Fadeel A.A.A., Abdel-Daim A.S.A., Abdel-Razik A.H., Youssef I.M.I. (2022). Effect of dietary protease supplementation on growth performance, water quality, blood parameters and intestinal morphology of Nile tilapia (*Oreochromis niloticus*). *J. Anim. Physiol. Anim. Nutr.*, 106: 419–428.
- Samtiya M., Aluko R.E., Dhewa T. (2020). Plant food anti-nutritional factors and their reduction strategies: an overview. *Food Prod. Proc. Nutr.*, 2: 6.
- Sánchez-Alcade M.C., García-Ulloa M., Montañó E.M., Castro-Martínez C., Álvarez-Ruiz P., Rodríguez González H. (2023). Use of enzyme mixtures in diets based on animal and plant ingredients for *Litopenaeus vannamei*: effect on digestibility, growth, and enzyme activity. *Turkish J. Fisher Aquat. Sci.*, 23: 21999.
- Sarraz Q., Hussain S.M., Sharif A., Selamoglu Z., Bashir F., Ahsan S. (2020). Potential of phytase supplemented *Moringa oleifera* leaf meal-based diet on mineral digestibility of *Oreochromis niloticus* fingerlings. *J. Surv. Fish. Sci.*, 6: 65–77.
- Shah S.Z.H., Fatima M., Afzal M., Bilal M. (2021). Interactive effect of citric acid, phytase and chelated mineral on growth performance, nutrient digestibility and whole-body composition of *Labeo rohita* fingerlings. *Aquac. Res.*, 52: 842–858.
- Sharawy Z.Z., Ashour M., Eman A., Ola A., Mohamed H., Hany N., Mahmoud K., Abdelwahab K., Mohamed H., Waldemar R., Ehab El-Haroun., Goda A.M.S-A. (2020). Effects of dietary marine microalgae, *Tetraselmis suecica*, on production, gene expression, protein markers and bacterial count of Pacific white shrimp *Litopenaeus vannamei*. *Aquacult. Res.*, 1–13.
- Shekarabi S.P.H., Ghodrati M., Masouleh A.S., Roudbaraki A.F. (2022). The multi-enzymes and probiotics mixture improves the growth performance, digestibility, intestinal health, and immune response of Siberian sturgeon (*Acipenser baerii*). *Ann. Anim. Sci.*, 22: 1063–1072.
- Sherine R.A., Hassaan M., Fitzsimmons K., El-Haroun E. (2023). Chapter 8. Alternative protein sources for sustainable tilapia farming. Novel approaches toward sustainable tilapia aquaculture. *Appl. Environ. Sci. Eng. Sust. Futur.*, https://doi.org/10.1007/978-3-031-38321-2_8
- Sherine R., Seyed H.H., Hien V.D., El-Haroun E. (2024). Evaluation of distillers dried grains with solubles in aquafeeds – a review. *Ann. Anim. Sci.*, 24: 65–75.
- Shi X., Luo Z., Chen F., Wei C.C., Wu K., Zhu X.M., Liu Z.X. (2017). Effect of fish meal replacement by *Chlorella* meal with dietary cellulase addition on growth performance, digestive enzymatic activities, histology and myogenic genes' expression for crucian carp *Carassius auratus*. *Aquac. Res.*, 48: 3244–3256.
- Shi Z., Li X.Q., Chowdhury M.A.K., Chen J.N., Leng X.J. (2016). Effects of protease supplementation in low fish meal pelleted and extruded diets on growth, nutrient retention and digestibility of gibel carp, *Carassius auratus* gibelio. *Aquaculture*, 460: 37–44.
- Soltan N.M., Soaudy M.R., Abdella M.M., Hassaan M.S. (2023). Partial dietary fishmeal replacement with mixture of plant protein sources supplemented with exogenous enzymes modify growth performance, digestibility, intestinal morphology, haemato-biochemical and immune responses for Nile tilapia, *Oreochromis niloticus*. *Anim. Feed Sci. Technol.*, 299: 115642.
- Song H.L., Tan B.P., Chi S.Y., Liu Y., Chowdhury M.A.K., Dong X.H. (2017). The effects of a dietary protease-complex on performance, digestive and immune enzyme activity, and disease resistance of *Litopenaeus vannamei* fed high plant protein diets. *Aquac. Res.*, 48: 2550–2560.
- Sriket C. (2014). Proteases in fish and shellfish: Role on muscle softening and prevention. *Int. Food. Res. J.*, 21: 433.
- Sureshkumar S., Song J., Sampath V., Kim I. (2023). Exogenous enzymes as zootechnical additives in monogastric animal feed: a review. *Agriculture*, 13: 2195.
- Terrey D.E., Braidi D.A., Serwata R. (2023). Effect of a microbial phytase on the growth performance, digestibility and retention in a high plant meal inclusion diet for Atlantic salmon (*Salmo salar*). *Aquacult. Int.*, <https://doi.org/10.1007/s10499-023-01295-1>
- Uzzau S., Bossi L., Figueroa-Bossi N. (2002). Differential accumulation of *Salmonella* [Cu, Zn] superoxide dismutases SodCI and SodCII in intracellular bacteria: correlation with their relative contribution to pathogenicity. *Mol. Microbiol.*, 46: 147–156.
- Velázquez-De Lucio B.S., Hernández-Domínguez E.M., Villa-García M., Díaz-Godínez G., Mandujano-Gonzalez V., Mendoza-Mendoza B., Álvarez-Cervantes J. (2021). Exogenous enzymes as zootechnical additives in animal feed: a review. *Catalysts*, 11: 851.
- Wang L., Zhou H., He R., Xu W., Mai K., He G. (2016). Effects of soybean meal fermentation by *Lactobacillus plantarum* P8 on growth, immune responses, and intestinal morphology in juvenile turbot (*Scophthalmus maximus* L.). *Aquaculture*, 464: 87–94.
- Wenxiang Y., Xiaoqin L., Kabir Chowdhury M.A., Jing W., Leng X. (2018). Dietary protease, carbohydrase and micro-encapsulated organic acid salts individually or in-combination improved growth, feed utilization and intestinal histology of Pacific white shrimp. *Aquaculture*, 503: 88–95.
- Wu J.J., Liu W., Jiang M., Zhou Y., Wang W.M., Wen H., Liu H. (2020). Beneficial effects of dietary exogenous protease on the growth, intestinal health and immunity of GIFT (*Oreochromis niloticus*) fed plant-based diets. *Aquac. Nutr.*, 26: 1822–1834.
- Wu J., Yu T., Wang Q., Zhang C., Fu D., Liu W., Jiang M., Xu L., Zhou Y., Wu J. (2024). Effects of dietary microbial protease on growth performance, nutrient apparent digestibility, hepatic antioxidant capacity, protease activities and intestinal microflora in

- juvenile genetically improved farmed tilapia, *Oreochromis niloticus*. Aquacult. Rep., 34: 101894.
- Yigit N.O., Bahadır Koca S., Didinen B.I., Diler I. (2018). Effect of protease and phytase supplementation on growth performance and nutrient digestibility of rainbow trout (*Oncorhynchus mykiss*, Walbaum) fed soybean meal-based diets. J. Appl. Anim. Res., 46: 29–32.
- Yuangsoi B., Klahan R., Charoenwattanasak S., Lin S.M. (2018). Effects of supplementation of pineapple waste extract in diet of Nile tilapia (*Oreochromis niloticus*) on growth, feed utilization, and nitrogen excretion. J. Appl. Aquac., 30: 227–237.
- Zamini A., Kanani H., Esmaili A., Ramezani S., Zoriezahra S. (2014). Effects of two dietary exogenous multi-enzyme supplementation, Natuzyme® and beta-mannanase (Hemicell®), on growth and blood parameters of Caspian salmon (*Salmo trutta caspius*). Comp. Clin. Pathol., 23: 187–192.
- Zarghi H., Golian A., Hassanabadi A., Khaligh F. (2022). Effect of zinc and phytase supplementation on performance, immune response, digestibility and intestinal features in broilers fed a wheat-soybean meal diet. Italian. J. Anim. Sci., 21: 430–444.
- Zhang Q., Li F., Guo M. (2023 a). Growth performance, antioxidant and immunity capacity were significantly affected by feeding fermented soybean meal in juvenile coho salmon (*Oncorhynchus kisutch*). Animals, 13: 945.
- Zhang Q., Guo M., Li F., Qin M., Yang Q., Yu H., Xu J., Liu Y., Tong T. (2023 b). Evaluation of fermented soybean meal to replace a portion fish meal on growth performance, antioxidant capacity, immunity, and mTOR signaling pathway of coho salmon (*Oncorhynchus kisutch*). Aquacult. Nutr., 2558173.
- Zheng C.C., Wu J.W., Jin Z.H., Ye Z., Yang S., Sun Y., Fei H. (2020). Exogenous enzymes as functional additives in finfish aquaculture. Aquacult. Nutr., 26: 213–224.
- Zhou Y., Yuan X., Liang X.F., Fang L., Li J., Guo X.Z., Bai X.L., He S. (2013). Enhancement of growth and intestinal flora in grass carp: The effect of exogenous cellulase. Aquaculture, 416–417: 1–7.

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