

# Improving the Quality of Feed Ingredients Using Enzymes

## *Meningkatkan Kualitas Bahan Pakan Menggunakan Enzim*

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### Abstract

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Commercial feed contributes to the operational costs of aquaculture production activities. On the other hand, making your feed requires formulation and availability of raw materials in maintained quantity and quality. Local feed raw materials can be used as alternative ingredients. Still, one of the obstacles is several limiting factors, such as crude fiber and the binding of some essential nutrients, such as protein and minerals. Hence, nutrient utilization is low and has a direct impact on growth. Adding feed additives as sources of enzymes is considered to help speed up the digestive process so sufficient nutrients are available for growth and survival. Enzymes break down large molecules such as crude fiber starch, increasing protein and fat into forms easily digested and absorbed by fish. This paper aims to review the use of enzymes to improve the quality of fish feed ingredients. The method used is Literature Review. Improving the quality of feed raw materials with the help of enzymes can increase the quality of fish feed ingredients. Application of enzymes in fish feed as additional feed ingredients (feed additives) through the addition of commercial enzymes, enzymes from plant or animal extracts, and microbial enzymes.

**Keywords:** Enzyme, Feed quality, Fish feed, Raw materials.

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### Abstrak

Pakan komersial memiliki kontribusi sebagai biaya operasional kegiatan produksi akuakultur. Di sisi lain, membuat pakan sendiri memerlukan formulasi dan ketersediaan bahan baku dengan jumlah dan kualitas yang terjaga. Bahan baku pakan lokal dapat dijadikan bahan alternatif, namun salah satu kendalanya adalah beberapa faktor pembatas seperti serat kasar, dan terikatnya sejumlah nutrient penting seperti protein dan mineral, sehingga pemanfaatan nutrient rendah dan berdampak langsung terhadap pertumbuhan. Penambahan *feed aditif* sumber enzim dinilai membantu dan mempercepat proses pencernaan, sehingga nutrient dapat cukup tersedia untuk pertumbuhan dan kelangsungan hidup. Enzim memecah molekul besar seperti serat kasar, pati, meningkatkan protein dan lemak menjadi bentuk yang mudah dicerna dan diserap oleh ikan. Tulisan ini bertujuan untuk mengulas pemanfaatan enzim untuk meningkatkan kualitas dari bahan pakan ikan. Metode yang digunakan adalah *Literature Review*. Perbaikan kualitas bahan baku pakan dengan bantuan enzim dapat memberikan peningkatan terhadap kualitas bahan pakan ikan. Aplikasi enzim dalam pakan ikan sebagai bahan pakan tambahan (*feed aditif*) melalui penambahan enzim komersial, enzim dari ekstrak tanaman atau hewan, dan dari enzim microbial.

**Kata kunci:** Enzim, Kualitas pakan, Pakan ikan, Bahan pakan

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## 1. Introduction

Feeding is one way to optimize aquaculture production, especially fish and shrimp (D'Abramo, 2021). Fish feed contributes to around 70-80% of the operational costs of aquaculture production activities. Feed ingredients are generally imported from animal feed ingredients, such as fish meal. For feed to have a price that suits farmers' ability, it can take advantage of several sources of vegetable feed ingredients because they generally feed ingredients quickly at affordable prices. Vegetable feed ingredients have a high crude fiber content (9-25%) and are rich in anti-nutritional substances that inhibit the digestive process in fish, so vegetable feed ingredients need to be processed first, some of which are fermentation methods, heating with high pressure, hydrolysis and soaking with solvents (Kallau, 2020, Samtiya et al., 2020)

Feed formulation consists of the primary raw materials as a source of protein and energy and feed additives for particular purposes. One specific goal is to improve the utilization of critical raw materials in formulations. Application of exogenous enzyme sources for fish obtained from outside the body of fish feed preparation before being given to fish. Enzymes are the most abundant class of proteins found in living cells and have an essential function as catalysts of biochemical reactions that collectively form the metabolism-intermediary of the cell (Wirahadikusumah, 2001). An enzyme can speed up a reaction  $10^8$  to  $10^{11}$  times faster than without a catalyst (Poedjiadi & Supriyatin, 2006). Enzymes are efficient and specific in their catalytic work, so enzymes are said to have very distinctive properties because they only work on particular substrates and specific reactions. With the presence of polar (or nonpolar) groups contained in the enzyme structure (Wirahadikusumah, 2001). Enzymes have advantages as biocatalysts with high specificity, accelerating chemical reactions and producing uncontaminated end products (Chaplin & Bucke, 1990). Processing feed ingredients using enzymes is a way to improve the nutritional value and quality of feed, primarily feed ingredients derived from waste. Giving enzymes to commercial feed can reduce crude fiber content from 10.65% (dose 0%) to 8.70% (dose 2.5%), can reduce nitrogen free extract content from 34.42% (0%) to 34.18 % (2.5%), and the organic material content has no effect (Wardani et al., 2014). Based on the description above, this journal review reviews the stability and types of enzymes and the results of improving the quality of the feed ingredients obtained.

The method used in this journal is the Literature Review method. A literature review is a description that discusses a theory, a finding, and critical research obtained with academic-oriented reference materials as a basis for research activities.

### *Fish Feed*

Feed is a critical aspect of aquaculture activities because feed is a source of energy for fish growth. The feed given must be available enough, and the amount shown is under the needs of the fish to grow because good feed suits the physiological demands and species of fish. Providing feed with good quality and quantity can optimize fish farming businesses (Fahmi et al., 2021). The types of feed ingredients are namely natural and artificial. Natural feed ingredients are feed ingredients that come from nature and have not gone through a complex processing process. Natural feed ingredients from various sources, depending on the animal type to feed. We will give natural plankton, worms, and insects in fish farming. According to Almaududy (2006), the advantage of artificial feed is that it has a nutritional content based on the dietary needs of fish and is more durable for the mouth opening of the fish.

Local feed raw materials that have potential as alternative feed raw materials are those derived from agricultural and plantation industry waste, such as oil palm meal, rubber seed meal, coconut meal, copra, and cocoa fruit skin, as well as livestock waste, such as rumen contents (Wizna et al., 2008), shrimp waste (Abun et al., 2021), chicken feather waste (Cortezi et al., 2008). Local feed raw materials have nutritional content with potential raw materials for animal and fish feed. Some local feed raw materials, such as palm kernel meal, have a protein content between 13.6-17.45% and crude fat content ranging from 17.1-21.55% (Sundu et al., 2010). Various processing methods for high-fiber ingredients have increased feed use efficiency, such as physical, chemical, and biological processing or its combination (fermentation). Enzymes can help break down large molecules such as crude fiber starch, increasing protein and fat into a form easily digested and absorbed by fish (Pamungkas, 2012). Enzymes in the feed can help speed up the digestive process so nutrients can be adequately available for the growth and survival of the cultivars.

### *The stability of the enzyme on the feed*

In addition to the possibility of species-related differences in the effectiveness of enzymes, it is also possible that the ingredients used in the diet determine whether an enzyme is effective or not. Adding 250 mg/kg of commercial protease preparations improved nutrient digestibility from a rainbow trout diet containing a mixture of canola and peas (Drew et al., 2005). However, the product did not positively affect other diets containing extruded flax and peas. Enzymes are added as supplements to feed to break down anti-nutritional factors included in the feed mixture. Most of these compounds are not easily digested by endogenous enzymes in the fish and can interfere with normal digestion (Wardani et al., 2014).

Other commonly expressed concerns about the effectiveness of enzyme applications in aqua feed include (a) The effect of temperature on the stability of enzymes applied in feed at the time in pellet machines or extrusion; (b) Enzymes in feed after thermal processing are coated, enzyme loss may also occur after placing feed in water, and (c) The effectiveness of microbial enzymes that have optimum temperatures at warm temperatures in cold-water animals that have low body temperatures (Poedjiadi & Supriyatin, 2006). The Aqua feed industry can adopt the widespread application of enzymes to improve feed performance. Given the low levels of enzymes in the gastrointestinal tract of aqua animals, feed additives must be added to increase feed efficiency.

## 2. Types of Enzymes

Several enzymes are divided based on substrate nutrients, remodeled types and their preparation as plant enzymes, and crude extract enzymes from rumen fluid enzyme based on nutrient substrate type:

**Protease.** Protease enzymes are prepared using *Bacillus cereus* and made inoculum by pouring enzyme liquid as much as 1,000 L<sup>-1</sup> into 1 kg of feed raw material mixture mashed and stirred evenly (Kurniasih et al., 2013). In addition, *B.subtilis* can be used inoculum through fish feed inoculated with 288×10<sup>5</sup> CFU/mL (10% inoculum), aerated, incubated at 37-40°C for seven days and water content (70% v/w) maintained by the addition of autoclaved water every day (Chaudhary et al., 2021). Protease enzymes can be used in fish feed to increase the availability of undigested protein and accelerate fish growth. Protease enzymes break peptide bonds in proteins into simpler molecules such as short oligopeptides or amino acids.

**Amylase.** Amylase enzymes can be prepared by adding rumen fluid by 620 and 1,240 U/kg to wheat pollard, reducing polysaccharide content by 4% and 3.9%, respectively, higher than that added enzymes (Pantaya et al., 2003). Crude enzymes are obtained from rumen fluid as slaughterhouse waste (RPH). The process includes centrifugation (10,000 rpm, 20 minutes at 4°C, and then the supernatant formed is reacted with 60% ammonium sulfate and stirred using a magnetic stirrer for approximately one hour. After precipitating for 24 hours, as much as 100 mL of rumen fluid supernatant dissolved in 100 mL of phosphate buffer pH 7.0 (ratio 1: 1) and stored at 4°C if not immediately used (Fitriliyani, 2010).

**Lipase enzyme.** Based on Mulia et al. (2015), lipase enzymes can be prepared with *R. Oligosporus* by fermentation processing. Lipase enzymes are enzymes that can break down fats into fatty acids and glycerol. This enzyme can be used in fish feed to increase the availability of undigested fat and accelerate fish growth.

**Cellulase enzyme.** According to Siregar et al. (2020), cellulase enzymes are added to *apu-apu* wood leaves by adding mold inoculum *Trichoderma harizanum* as a fermenter. *Apu-apu* leaf flour is first added to water as much as 0.5 L<sup>-1</sup>, then for 45 minutes, cooled and inoculated with *T.harizanum* culture at a dose of 7%, packaged and stored in aerobic conditions. After 18 hours of the fermentation process, leaves are steamed for 45 minutes to stop the fermentation process, cooled, and mashed into flour. Cellulase enzymes can also be prepared by fermentation of ingredients, namely “onggok” (cassava waste), through *A.niger* fungi as fermenters (Winantu, 2018).

### *Plant Extract Enzymes*

**Papain enzyme.** Papain enzymes result from extracting papaya fruit, which is used as a powder as a feed additive (Permata et al. 2019). Papain is a protease enzyme found in papaya sap. These enzymes are used to hydrolyze peptide bonds in proteins. Adding papain to the feed promotes catfish growth in a relatively short time. Enzyme preparation from papaya fruit sap added NaCl can activate the papain enzyme and then dried and added 2.25% coarse papain, in formulation (fish meal 39%, soybean 32.25%, corn 4.25%, and rice bran 6.75%, wheat flour 10%, premix 4%, and carboxy-methyl-cellulose 1.5%) (Kusumadajaja & Dewi, 2005). The resulting pellets smell almost the same as catfish feed on the market, but the shape was not yet uniform. An optimal dose of 2.53% papain enzyme on artificial feed can produce a maximum relative growth rate of 5.05%/day for catfish (*Clarias gariepinus*) (Khodijah et al., 2015).

**Bromelin enzyme.** Bromelin or bromelain, an enzyme in pineapple, is proteolytic, which can hydrolyze proteins into their constituent elements. According to Putri (2012), hydrolysis occurs when proteolytic enzymes break peptide bonds from substrate bonds, where proteolytic enzymes serve as cell catalysts. Bromelin enzyme can act as an exogenous enzyme. Bromelin enzyme can also dissolve collagen in collagen protein by hydrolyzing the protein. Soybean meal resulting from hydrolysis by 10% pineapple fruit extract, produces better fish growth compared to hydrolysis of a mixture of fish meal and soybean meal.

### *Phytase Enzyme from Microbes*

Probiotics and prebiotics-supplemented feeds mediate intestinal microflora to secrete major enzymes such as amylase to stimulate digestion (Chaudhary et al., 2021). Phytase is an enzyme capable of hydrolyzing phytate into inositol and phosphoric acid. Phytic acid will reduce the body's ability to absorb minerals and proteins (Anggani et al., 2021). Phytic acid can reduce the digestibility of nutrients contained in artificial feed. Reducing phytic acid content in artificial feed can be done by adding exogenous phytase enzymes (Rachmawati & Samidjan, 2014). The phytase enzyme can convert anti-nutrition phytate substances into inositol and phosphoric

acid so nutrients can be digested properly by cantang grouper (*Epinephelus* sp). Some commercial enzymes and sources of mushroom extracts, including:

***Aspergillus niger***. According to Zulaeha et al. (2015), the phytase enzyme brand "Nathupos 5000@" is derived from the fungus *A.niger*, which is given into feed ingredients for bran flour, soybean flour, corn meal, bran flour, and fish meal. Phytase enzyme treatment doses of 0, 0.5, 1.0, and 1.5 g/kg feed gave the best results at 1.0 g/kg in cantang grouper (*Epinephelus* sp). (Chrisdiana et al., 2015; Zulaeha et al., 2015).

***Peniophora lycii***. The phytase enzyme is granules made from *P.lycii* mushrooms fermented with *A. oryza* fungi. The particle size is 600 µm, and the color of the fungal colony varies, while the outer layer is brownish with small grains, not dusty (Rochmawati, 2016).

### 3. Application of Enzymes in Fish Feed

The use of enzymes in the processing of fish feed raw materials is a breakthrough effort practically used in feeding. Enzymes can function outside living cells as biological catalysts in vitro. Enzymatic activity is related to protein structure because enzymes have an active side that binds to the substrate, the typical source of enzymes from digestive and exogenous enzymes. In general, digestive enzymes are pure protein structures such as the uricase, pepsin, trypsin, and chymotrypsin. Exogenous enzymes are obtained from outside the body and are usually applied in raw material processing, as seen in Table 1.

Table 1. Some applications of enzymes in fish feed

Types of Enzymes	Enzyme preparation	Raw materials	Quality improvement results	Reference
Protease	<i>B. cereus</i> made inoculum	Local ingredients	Increase protein retention and palatability of formulated feed	Kurniasih et al. (2013)
Amylase	Hydrolyzed rumen fluid	wheat pollard	Reduced polysaccharide content by 3.9- (without enzymes) and 4% (added enzymes)	Pamungkas (2012)
Lipase	Inoculum <i>R.oligosporus</i>	Tofu pulp and rice bran	Decreased fat content by 13.33%	Mulia et al. (2015)
Protease and Phytase	<i>B. subtilis</i>	Commercial fish feed	Inoculum dose 10% ( $288 \times 10^5$ CFU /mL). Effectiveness, growth, and increased protein efficiency with low FCR	Chaudhary et al. (2021)
Papain	Papaya fruit extract	Pellets with fish meal, soybeans, bran	Crude protein 30.22%, crude fat 10.48%, nitrogen free extract 30.73%, an energy value of 267.49 (kcal/g), an energy-protein of 8.85 (kcal/g), and increased catfish growth.	Permata et al. (2019)
Bromelin	Pineapple	Bran, soybean, bran, corn flour	The addition of bromelin enzyme in artificial feed has a noticeable effect on the efficiency of feed utilization.	Putri (2012)
Cellulase	<i>T. harzianum</i>	Substituting soybean with <i>Pistia stratiotes</i>	Substituting soybean flour at a rate of 50% with dry matter and protein digestibility (76.7%; 86.9%), feed efficiency of 44.50%, protein retention of 51.94%, specific growth rate of 4.49% and survival rate of 96%	Siregar et al. (2020)
Protease	<i>A.oryzae</i>	Coconut peel	The addition of fermented coconut pulp to the feed is optimal for growth and protein content of tilapia by 25%	Elyana (2011)
Phytase	<i>A.niger</i>	A mixture of rice bran, soybeans, fish meal	The phytase dose of 1,000 mg/kg of feed shows the best results, utilizing feed well to make feeding more efficient.	Chrisdiana et al. (2015)
Phytase	<i>P. lycii</i>	Commercial fish feed	The phytase dose of 1,000 mg/kg of feed increased the specific growth rate and ratio of feed efficiency and protein efficiency ratio of tilapia ( <i>O. niloticus</i> ) fry, with digestibility values of crude protein and a total of 84.88% and 71.27%.	Rachmawati & Samidjan (2014)

Types of Enzymes	Enzyme preparation	Raw materials	Quality improvement results	Reference
Cellulase	<i>Bacillus amyloliquifaciens</i>	cassava waste	Increase crude protein by up to 360% and reduce crude fiber content by 32%	Wizna et al. (2005)
Phytase	Fitase“Nathupos 5000®”	soybean meal	A dose of 986 mg/kg feed increased the relative growth rate by 2.24%, with feed efficiency at 950 mg/kg in duck grouper.	Zulaeha et al. (2015)
Enzyme cellulase	Actinomycetes molds	lignocellulose material	Improve the degradation process of lignocellulolytic material into feed raw materials that fish can digest.	Suryaningrum & Samsudin (2019)

## 4. Conclusions

Improving the quality of feed raw materials with enzymes has several significant benefits in feed processing. Enzymes have types; the origin is obtained, and the results of increasing feed ingredients vary from each type. Further application research and standardization of enzyme preparations are needed to get enzymes in the commercial direction.

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