## Stability improvement of aqua feed enzymes

Ajay Guru<sup>1</sup>, Nithya S<sup>2</sup>, Prasanna Devi D<sup>2</sup>, Pongomathi K<sup>2</sup>, Kasi Viswanathan<sup>3,4</sup>& Jesu Arockiaraj<sup>1,2\*</sup> <sup>1</sup> SRM Research Institute, SRM Institute of Science and Technology, Kattankukathur 603 203, Chennai, Tamil Nadu, India <sup>2</sup>Foundation for Aquaculture Innovations and Technology Transfer (FAITT), Sanigudem Cross Road, Galavagudem 534 450, DenduluruMandalam, West Godavari District, Andhra Pradesh, India 3Biogreen Technologies, Sai Madhu Arcade, Telecom Nagar, Vatluru, Eluru 534 007, West Godavari District, Andhra Pradesh, India <sup>4</sup> Aguatics International, Ganesh Apartment, Senthil Nagar, Perungudi, Chennai 600 096, Tamil Nadu, India

\* Corresponding author: Dr. Jesu Arockiaraj, Professor, DBT-Ramalingaswami Fellow, Room No. 201, II Floor, Sir C.V.Raman Research Park, SRM Research Institute, SRM Institute of Science and Technology, Kattankulathur, Chennai 603 203. Tamil Nadu, India, Email: jesuaroa@srmist.edu.in

### **Summary**

The addition of exogenous enzymes in aquafeed may improve the growth efficiency of the organisms by improving the digestibility of nutrients and increasing the activity of the digestive enzyme. A major concern in the use of enzymes in aquatic feed is their stability under different processing conditions, including heat, pressure, and humidity. This report provides some useful information on maintaining the enzyme activity or how to avoid enzyme activity reduction which passes through the extrusion process.

Keywords: Feed, Feed enzyme, Enzyme activity, Enzyme stability, Temperature

#### Introduction

Plant food is relatively cheaper than fishmeal, although it is similar in protein. The existence of an anti-nutrition factor is one of the major problems faced by the use of plant products in aquafeed.Dietary fibers are non-digestible carbohydrates including pectin and cellulose. For monogastric animals such as fish, their digestibility is very low. A potential solution to this problem is to increase the use of the exogenous enzyme. It is therefore necessary to find ways to improve the availability of digestive enzymes in extruded fish or shrimp feeds. The report revealed the various enzymes that can be incorporated into aquafeed and the possibility to maintain its stability which passes through the pelleting temperature.

#### Importance of exogenous enzymes in aquafeed

Feeding enzymes for shrimps and fish is one of the major nutritional breakthroughs in the aquaculture sector in recent years. Exogenous enzymes are now widely used as additives in animal diets around the world. Also, enzyme supplementation can help to reduce the effects of antinutritional factors and increase the use of dietary energy and amino acids, resulting in improved animal performance (Fig. 1 & Table 1). Enzymes provide additional powerful tools that can inactivate anti-nutritional factors and enhance the nutritional value of plant-based proteins in food. They have a natural way of transforming the complex components of the feed into absorbable nutrients. Adding enzymes in the diet increases the use of nutrients, reduce the cost of feed and excretion of nutrients in the



Fellow jesuaroa@srmist.edu.in

system. Enzymes develop from higher animals and plants to the simplest unicellular forms of life in any living organism, as they are essential for the metabolic cycle.

#### **Extrusion process**

Aqua feed extrusion process refers to cooking a mixture of feed ingredients under high temperature, humidity and high pressure within a short time using a feed extruder, where high temperature is a direct result of friction. As a technological treatment, extrusion may allow the processing of a variety of feed ingredients, such as high water content of soybean, corn, rice, peas, and raw materials. The extrusion of aqua feed can be divided into two types, depending on the raw material content of the treatment. They are dry type extrusion and wet type extrusion.

1. The dry type extrusion process adopts heat from friction for heating materials, forces the materials to pass through the die hole and, at the same time, the action of the screw extrusion is subject to a certain pressure. The pressure of the materials decrease sharply after extrusion out of the die hole and the moisture evaporates to achieve the goal of extrusion. Water content is always around 15% to 20% throughout this process.

- 2. Wet type extrusion processes the concept is similar to dry type extrusion processes.
- Other than the dry type, the wet type feed extruder system is fitted with a boiler that can continuously add vapor to the chamber while extruding feed ingredients, making floating pellets.

### Advantages of extruded feed

The basic function of extruded feed produced through the extrusion process is to kill germs in food ingredients, improve quality and provide safe food for the aquatic animals. Also, there are other major benefits of extruded aquafeed:

- The extruded feed can float on water for a long time, making it easier to monitor the ingestion of fish to change the quantity of food on time and to manage the growth and health of fish.
- In the extruded process, heat, steam, pressure and other mechanical action may promote the degree of starch pasting, break and soften the cell wall of the fiber structure and release certain digestible materials that favor digestion and absorption of aquatic livestock.

### Enzymes available in the commercial market

Liquid and dry commercial formulations of feed enzymes are available in the global market. Due to factors such as ease of handling, good thermal stability during feed processing and advanced enzyme activity, these products are strongly preferred over liquid-based additives.

Dry formulations are also available in the global market as both powder and pellet grades, which is less attracted due to the powder or pellet grades may not be evenly mixed or distributed in the feed. However, dry poweder or pellet grade enzymes have higher chance of being thermal stable passes through the pelleting or extrusion temperature.

### Powder enzyme

Most feed enzymes are used in powder or granular form, which can be applied for both farm application and feed incorporation. Food conditioning (e.g. pelleting, expansion, extrusion) may adversely affect the efficacy of powdered enzymes, as these enzymes are rapidly inactivated at temperatures above 50–60 °C. e.g., For example, a protease enzyme is produced using cultivation and extraction techniques from *Bacillus licheniformis*. This product contains abundant protease, which can hydrolyze peptide bond protein that binds with an amino acid to adequately release plant protein.

#### Liquid enzyme

The post-pellet spray with liquid enzyme provides an alternative to the addition of powder enzymes before conditioning. The use of liquid enzymes means less handling, more processing, and there is very little chance of people coming into contact with the enzyme. The addition of liquid enzymes is also cheaper, as it requires less amount compared to powder enzyme. In the case of powdered enzymes, the dosage was approximately 600 to 1000 gof feed per tonne. Only half of this amount is required for the liquid enzyme. For example, liquid enzyme (composed of cellulase, xylanase, protease, alpha-amylase, pectinase, lipase and mannanase) leads to easy digestibility.

### Benefits of exogenous enzymes

The application of exogenous enzymes in aquafeed has the following benefits on the fish or shrimp:

- Reduces viscosity of the digest
- Enhances the digestive and absorption of nutrients, particularly fat and protein.
- Increases feed consumption, weight gain and feed gain ratios

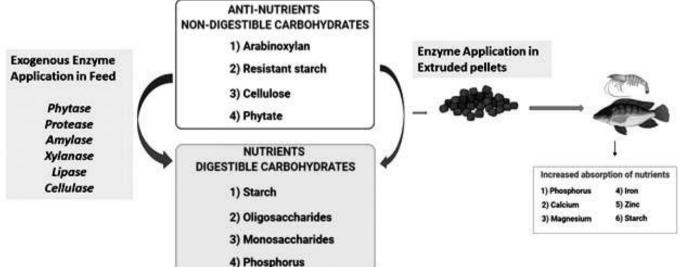


Fig. 1. A process explaining the exogenous enzyme application in extruded pellets which have the nutritional benefits to the aqua organisms.

Table 1. Various exogenous enzyme application in aquafeed and their benefits on the aquaculture organisms

Enzyme	Function
Phytase	It helps to break down phytate phosphorus which allows for better absorption of phosphorus by monogastric
	animals and reduces the need for additional supplements of calcium phosphate
Protease	Essential for digestion of proteins in plants and animals
Amylase	Amylase is responsible for breaking the bond into simpler sugars in starch, polysaccharides and complex
	carbohydrates
Xylanase	Xylanase hydrolyzesxylan and arabinoxylan, the main component of hemicellulose
Lipase	It is necessary for the complete digestion of fats to their smaller fatty acid components
Cellulase	Cellulases break down the cellulose molecule into monosaccharides such as beta-glucose or shorter
	polysaccharides and oligosaccharides

- Improves digestibility of nutrients
- Reduces production of ammonia

### Phytase

Phytases have a maximum temperature range from 45°C to 60°C (113 to 140°F). Phytases may be partially or completely inactivated by overheating or by high temperatures for steam pelleting. To avoid this, the temperature of the extruder feeding section was maintained by circulating water through the jacketed device, the temperature of the metering and die section were both maintained at that corresponding to each run using the extruder's heat control device.

### Protease

The complete inactivation of the protease occurred at 100°C, irrespective of the extruder screw speed and setup. To overcome this issue, feed ingredient and protease mixture was granulated into sinking pellets by an extruded after oil and water were added to obtain a homogeneous mixture. The pelleting temperature

and the extruder temperature were  $80 \pm 5$  °C. All diets had a size of approximately 2.0 mm diameter and were air-dried and stored at 4 °C until further use

### Amylase

The optimal temperature for the enzymatic activity of amylase ranges from 32 °C to 37 °C. When the optimum temperature exceeds, the amylase break starch very quickly. At low temperatures, amylase slowly breaks down the starch due to reduced kinetic energy. So that amylase enzyme supplements were added to the diets in a freeze-dried nylon-protein microencapsulated form.

The dry feed ingredients were thoroughly mixed with an equivalent amount of distilled water, which had a pH of 6.8-7.0, adjusted with 25% NaOH additions. The microcapsules were evenly dispersed in the dough like substance mixed by hand and extruded. The feeds were allowed to dry overnight at 40°C and stored in sealed plastic bags at 5°C.



### Xylanase

Xylanase enzyme has an optimal activity level at 50 °C with a pH value of 5. Xylanase suffered a large drop in activity level and was even completely inactivated at 90 °C. So to come over that, Xylanase and aqua feed were thoroughly mixed to turn them into a stiff dough. A hand-noodle marker was used to extrude the dough. A cooling wet paper tower continuously cooled the unit. The diets were fan-dried immediately at room temperature. The pellets were then deposited at -20 °C after drying.

### Lipase

The heat increment of oil is lower than carbohydrates and protein which reduces the consumption of unnecessary heat dissipation. Temperature stress can cause the activity of the enzyme to decline, so adding oil is good for fish feed. Spraying the oil increases the physical property and palatability of pelleted feed. Feeds have been formulated to contain 25% lipid following commercial requirements for high-energy diets. The lipase was made up of protein and the starch carrier, in powder form. For each form of feed, the correct amount of enzyme was mixed in fish oil by gently stirring for 10 min and then applied to feed before extrusion.

### Cellulase

The optimal temperature for the stability of the cellulose enzymes ranged from 50 to 55 °C. Soybean meal helps to maintain pelleting consistency during high flow temperatures.

# Aquaculture's role in nutrition in the COVID-19 era



A new paper from American University published examined the economics of an aquaculture industry of the future that is simultaneously environmentally sustainable and nutritious for the nearly 1 billion people worldwide who depend on it for health and livelihoods."The paper's primary author Jessica Gephart said "Seafood is essential to meeting global food and nutrition security goals," said Jessica Gephart. So the soya bean oil was gradually added as the mixture continued, and all the ingredients were added together for another 10 min. In the end, distilled water was added to form a dough. A pelletizer with a 2 mm diameter die was passed through an extruder. The diets were dried at 55 °C overnight and the dry pellets were put in sealed plastic bags and stored at 20 °C until they were used.

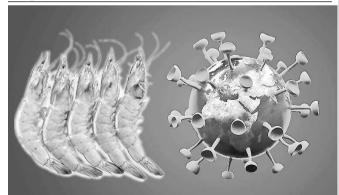
## Exogenous enzymes stability maintenance in pelleting temperature

Feed ingredient price has influenced through the use of an exogenous enzyme to increase nutrient quality in diets. If exogenous enzymes are to be added before pelleting at the mixer, then pelleting variables that increase feed heat and pressure can denature exogenous enzyme components, decreasing the efficacy of exogenous enzymes. However, the primary pelleting temperature was modified to reduce the loss of the exogenous enzyme to the feed.

### Conclusion

Enzymes can, therefore, play an important role in the production of eco-friendly aqua-feeds and help to reduce the need for fishmeal. The use of enzymes as a feed additive has grown rapidly and has been an effective tool for use in aquafeed. Although the economic and social benefits of enzymes have been well established, the future of feed enzymes is a bright one for the aquaculture industry.

China customs authority announced that it suspends imports of Ecuador shrimp on Covid-19 pandemic risk



Customs authority said samples taken from shipments from Industrial Pesquera Santa Priscila SA, Empacreci SA and Empacadora Del Pacifico Sociedad Anonima Edpacif had produced six positive results. However, tests on the frozen shrimp and inner packaging were negative. Earlier China began testing imported fresh and frozen food after the coronavirus was found on a chopping board used to cut salmon at a large food market in Beijing.