



## Is shrimp physiology and productivity enhanced by Bile acids?

H. Manimaran<sup>1</sup>, S.Athithan<sup>1</sup>, P. Chidambaram<sup>1</sup>, Joshna.M<sup>1</sup>

<sup>1</sup>Tamil Nadu Dr. J. Jayalalithaa Fisheries University-Nagapattinam- 611 002.

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### Introduction

The primary supply of aquatic food now comes from shrimp farming rather than fisheries. It is a prosperous sector that supports many people's livelihoods globally and generates revenue and employment. Since shrimp is high in protein, vitamins, minerals, and antioxidants, it is greatly sought after for its nutritional value and health advantages. Because shrimp has a high economic value in the production and supply chain, there is a need for them. In spite of obstacles such as viral infections, efforts are being undertaken to enhance hatchery biosecurity and health management in order to guarantee the generation of healthy post larvae. Feeding and nutrition will be crucial to the continued advancement of shrimp growing techniques. Regardless of the culture system in which they are raised, shrimp and other aquatic creatures depend primarily on an adequate supply of nutrients, both in terms of quantity and quality. (Marvasti and Carter, 2016). Input supply (feeds, feed additives, etc.) must be guaranteed in order to meet the nutrient and energy needs of the species being cultivated as well as the system's production objectives. Exogenous bile acid supplementation can significantly

increase the digestion and utilization of fat in shrimp diets by giving the fat additional energy (Daniel, 2016). Bile acids reduce feed costs and enhance growth performance, based on the multiple physiological functions of bile acids in animals. Bile acids have been authorized as a new feed additive in China, garnering increasing attention in the aquaculture industry since 2014 (Yao et al., 2021).

### Feed additives' roles in the nutrition of shrimp

In Aquaculture production, feed additives have a vital role in improving feed efficiency and more importantly, in achieving low feed conversion ratios (FCRs). Feed additives can act on an aquatic animal's system directly or indirectly, they can contain both nutritive and non-nutritive components. Small amounts of feed additives are added with the specific goal of raising the quality of shrimp as a finished good. Additionally, it maintains the aquatic environment's quality and the physical, chemical integrity of the food. The feed additives' capacity to accelerate growth or enable the species to achieve its maximal sizes has been described. Feed additives have been



essential to aquaculture enterprises in order to support ideal growth development and survival within a given time period. To maintain the nutritional qualities of a diet or feed materials before feeding, additives are employed in aqua feed. The feed additives function as grow promoters, molting inducers, emulsifiers, stabilizers, binders, feed stimulants, probiotics, prebiotics, antibiotics, mycotoxin, antioxidants, color/pigmentation agents, antimicrobial compounds, disease preventer, organic acids, bile acids, hormones, and herbal extracts, among other things. Enzymes, such as proteases and amylases, are also utilized to increase the availability of specific nutrients or to reduce the presence of specific anti-nutrients, such as phytase. The feed additives help cultured organisms develop and function better in terms of health. Moreover, feed additives strengthen their defenses against illness and stimulate bodily functions outside of regular feeding.

#### **Bile Acids as Feed Additive**

The primary component of bile is bile acids, which are made in the liver. Bile acids are released into the colon, where they provide vital biological functions such as protecting animal liver, enhancing fat usage as a natural emulsifier, and activating lipase to promote fat digestion and it has antimicrobial properties (Merritt and Donaldson, 2009). As the primary source of energy for animals, fats and oils have about three times the apparent metabolizable energy of other feedstuffs and the highest caloric value of all nutrients. As a result, lipids are frequently added to animal diets in order to satisfy energy needs. Because their bile secretion is limited, young animals have inadequate development of their ability to digest and absorb dietary fat. In recent years, bile acids are widely used

as feed supplements in aquaculture (Gu et al., 2017).

#### **Bile acids**

An essential physiological component for intestinal nutrient absorption is bile acids. The byproducts of the breakdown of cholesterol are bile acids. The production of bile acids leads to the flow of bile and the discharge of phospholipids, cholesterol, medicines, and toxic metabolites. The primary active element in bile is bile acids. Bile acid also has a variety of biological actions as it is made up of complicated components. Bile acids help with the solubilization, emulsification, and metabolism of fat and fat-soluble vitamins (Romano et al., 2020) and safeguards fish and shrimps' vital organs. Because bile acids act as a detergent on dietary fat particles, fat globules disintegrate or emulsify into tiny, microscopic droplets. Although emulsification does not constitute digestion, it does significantly increase fat's surface area so that lipases can break it down.

Bile acids are lipid transporters that can solubilize a wide range of lipids by aggregating fatty acids, cholesterol, and monoglycerides into micelles (Di Ciaula et al., 2018). Additionally essential for the movement and assimilation of fat-soluble vitamins are bile acids. Through FXR binding to response element-binding protein (SREBP-1C), bile acids can control the metabolism of hepatic triglycerides in the liver and lessen hepatic steatosis (fatty liver). Bile acids also increase the synthesis and transit of low-density lipoprotein (LDL) (low-density lipoproteins) and VLDL (very low-density lipoproteins) and lowers plasma cholesterol and triglyceride levels.

#### **Mechanism of Bile Acids**



The primary active ingredient in bile are a group of sterols that are created during an animal's process of metabolism of cholesterol known as bile acids. Bile acid also has a variety of biological actions and is a complicated blend of components. The solubilization, emulsification, and use of fat and fat-soluble vitamins are the main purposes of bile acids. It safeguards the liver, hepatopancreas of shrimp. The detergent effect of bile acids on dietary fat particles leads fat globules to disintegrate or emulsify into tiny, microscopic droplets. Although emulsification does not constitute digestion, it does significantly increase fat's surface area so that lipases can break it down. Bile acids are lipid transporters that can solubilize a wide range of lipids by aggregating fatty acids, cholesterol, and monoglycerides into micelles. The transportation and absorption of the fat-soluble vitamins depend on bile acids as well.

#### **Importance of bile acids in shrimp**

Lipids are the organic reserves found in shrimp and other crustaceans. They are often the second largest biochemical fraction in these animals, behind protein. The primary lipid found in shrimp is cholesterol, which can be found alone or in conjunction with fatty acids in all of the cells and the hemolymph. Shrimp and other crustaceans are unable to secrete cholesterol and bile acids. They need cholesterol to transform into a hormone that facilitates molting and helps them move quickly through the many stages of larval development. For shrimp, the state of the hepatopancreas is critical and directly influences the survival rate.

#### **Role of hepatopancreas in Shrimp**

Shrimps, along with other arthropods, mollusks, and fish, have a hepatopancreas (HP), or digestive gland, which performs

the same functions as the liver and pancreas in mammals. It serves as the primary organ for the storage of fats, glycogen, and many minerals as well as the transportation, secretion of digestive enzymes, and absorption of food (Diaz et al. 2010). The HP has many blindly terminating tubules that are kept together loosely by basophilic connective tissue strands. The HP is surrounded by a thin membrane of connective tissue, which increases the surface area available for absorption and processing. The lumen in the center of each HP tubule is lined by epithelium. The HP tubule epithelium has five cell types that have been identified as being involved in digestion. Four of them are described (Felgenhauer, 1992; Bell and Lightner, 1998).

#### ❖ **E-cells (embryonic or Embryozellen)**

The other three cell types of the digestive gland are derived from these structures, which are located at the distal tips of each tubule and have prominent nuclear bodies and proximal nuclei.

#### ❖ **R-cells (resorptive/absorptive or Restzellen)**

Multi-vacuolated cells are found all over the HP and are used for lipid and glycogen storage as well as absorption. Additionally, they frequently trap mineral deposits that contain sulfur, calcium, magnesium, phosphorus, and other elements.

#### ❖ **B-cells (blister-like or Blastozellen)**

Large, primary secretory cells are in charge of intracellular digestion, nutrition accumulation, and the transportation of digested matter. They are also the principal producers of digestive enzymes in the HP.

#### ❖ **F-cells (fibrillar)**

F-cells (fibrillar) are responsible for protein synthesis and storage of minerals.



### **Bile Acids' Role in Shrimp Feed**

The hepatopancreas is suffering from various health issues, including significant degradation of the pond environment, petroleum product residue, heavy metals, ammonia nitrogen, disinfectant residue, and more. Furthermore, a high protein and fat composition places a significant strain on the enterohepatic system. Diseases in shrimp are caused by unfavorable environmental factors, bacterial infections, and weakened immune systems. The bile salt helps the hepatopancreas perform a variety of tasks in *Penaeus vannamei* by acting as a potent protective agent. The utilization of bile acid as a feed supplement is crucial for improving existing management techniques toward sustainable shrimp farming and for increasing better growth and solving numerous issues.

### **Fat digestion and absorption**

Through fat emulsification, lipase enzyme activation, and the formation of mixed chylomicrons with enzymatic hydrolysate, bile acids facilitate the digestion and absorption of fat and cholesterol. Fat Digestion in shrimp occurs in the hepatopancreas, which serves as both the digestive gland and the liver. The process involves:

- **Lipase Enzymes:** The hepatopancreas secretes lipases, enzymes that break down triglycerides (fats) into fatty acids and glycerol.
- **Emulsification:** Lipoproteins (proteins coated with lipids) bind to fatty acids and glycerol to form micelles, small emulsion droplets.
- **Micelle Absorption:** Micelles pass through the digestive tract and are absorbed into the hepatopancreas cells.

- **Intracellular Digestion:** Fatty acids and glycerol are further broken down and re-synthesized into triglycerides and other lipid components.

Bile acid are synthesized in the hepatopancreas and play a crucial role in fat digestion and absorption. They:

- **Emulsify Fats:** Bile acids act as detergents, helping to disperse fats and form micelles.
- **Stimulate Lipase Activity:** Bile acids activate digestive lipases, enhancing fat digestion.
- **Absorption:** After digestion, bile acids are reabsorbed from the digestive tract and returned to the hepatopancreas. This process is known as the enterohepatic circulation.

Fat and bile acid metabolism in shrimp is regulated by hormonal and environmental factors:

- **Hormones:** Insulin-like growth factor (IGF) and ecdysteroids promote the synthesis and secretion of bile acids and digestive enzymes.
- **Temperature:** Higher temperatures increase the activity of digestive enzymes and bile acid secretion.
- **Diet:** A high-fat diet stimulates bile acid production and fat digestion.

Adequate fat and bile acid absorption are essential for shrimp. Fats provide energy for shrimp growth and metabolism. Bile



acids are necessary for the synthesis of steroid hormones involved in reproduction. Bile acids aid in the absorption of fat-soluble vitamins (A, D, E, K).

### **Lipid Metabolism**

Bile acid is crucial for controlling the metabolism of fats, carbohydrates, and energy. Bile acid as a chemical that signals to control the metabolism of fats. Numerous nuclear receptors in the gastrointestinal system and liver are activated by bile acid. The main mechanism for the breakdown of cholesterol is bile acid production. Bile acids form micelles that emulsify dietary fats, breaking them down into smaller droplets. Bile acids bind to fats, forming water-soluble complexes that can be absorbed by the intestinal epithelial cells. Bile acids aid in the excretion of excess cholesterol from the body. Bile acids are synthesized from cholesterol through a multi-step process that involves enzymes known as cytochrome P450 enzymes. Conversion of cholesterol to  $7\alpha$  hydroxycholesterol. Oxidation of the side chain of  $7\alpha$  hydroxycholesterol. Conjugation of the bile acids with amino acids, glycine or taurine, to form bile salts. Bile salts are excreted into the bile, which is stored in the hepatopancreas.

Bile salts are released into the intestine during digestion, where they assist in lipid absorption. After absorption, they are reabsorbed in the lower intestine and transported back to the hepatopancreas for reuse. The synthesis and release of bile acids in shrimp are regulated by various factors; High dietary lipid intake stimulates bile acid synthesis. Hormones such as ecdysone and insulin-like growth factor (IGF) promote bile acid synthesis. The gut microbiome can influence bile acid

metabolism by producing enzymes that modify bile acids.

Effects on lipid metabolism improve the absorption of dietary fats, leading to increased energy intake. Bile acids can affect the expression of genes involved in lipid metabolism, including those involved in fatty acid synthesis and oxidation. Bile acids help maintain cholesterol homeostasis by facilitating the excretion of excess cholesterol. Bile acids play a vital role in lipid metabolism in shrimp by emulsifying dietary fats, promoting fat absorption, and regulating cholesterol homeostasis. Their biosynthesis, excretion, and circulation are regulated by various factors, ensuring efficient utilization of dietary lipids. Understanding bile acid metabolism can help optimize shrimp production and improve their nutritional value.

### **Enhance the Immunity of Shrimp**

When fed continuously, bile acids can make shrimp more resistant to disease. In the gastrointestinal tract, bile acids aid in establishing an environment that kills and breaks down specific bacteria and viruses. Feeding shrimp bile acid can help them function as a better hepatopancreas and strengthen their immune systems.

- **Antimicrobial activity:** Bile acids have direct antimicrobial activity against various pathogens, including bacteria and viruses. They can disrupt the integrity of microbial membranes, leading to cell death.
- **Immunomodulation:** Bile acids can modulate the immune response of shrimp by regulating the expression of immune genes and the production of immune mediators. For example, they can stimulate the



production of antimicrobial peptides and cytokines.

- Gut barrier function: Bile acids contribute to the maintenance of gut barrier function by regulating the composition and diversity of the gut microbiome. A healthy gut microbiome is essential for preventing the colonization and invasion of pathogens.
- Immune cell activation: Bile acids can activate immune cells, such as hemocytes, and enhance their phagocytic and killing abilities.

Specific examples of bile acids enhancing immunity in shrimp:

- White spot syndrome virus (WSSV): Bile acids have been shown to inhibit the replication of WSSV in shrimp and reduce mortality rates.
- White fecal Syndromes (WFS): The hepatopancreas becomes infected with bacteria from many sources, leading to White Fecal Syndromes (WFS) and White Gut syndrome in shrimp. When bile acids are consistently added to a shrimp's diet, the hepatopancreas is shielded from the basic cause of the shrimp's WFS.
- Prevents Hepatopancreas Necrosis (EMS/HPNS): Shrimp hepatopancreas necrosis is directly caused by *Vibrio parahaemolyticus*, toxic algae, and detrimental pond physicochemical conditions. This disease is also caused by the following elements: toxic algae, low stress resistance, excessive pathogen load, and problems of the ecological agricultural system. By regularly providing shrimp with bile

acid, the hepatopancreas can be shielded from infections.

- Immune gene expression: Bile acids have been found to upregulate the expression of immune genes, such as those encoding antimicrobial peptides and cytokines, in shrimp. Bile acids play a multifaceted role in enhancing the immunity of shrimp. Their antimicrobial activity, immunomodulatory effects, and contributions to gut barrier function and immune cell activation help protect shrimp from pathogens and maintain their overall health. Understanding the role of bile acids in immunity can lead to the development of novel strategies to improve shrimp disease resistance and aquaculture practices.

#### **Prevents Toxin Compounds**

Bile acids have the ability to break down intestinal endotoxin and lessen the harmful chemicals on the hepatopancreas. It lowers intestinal endotoxin absorption and stops endotoxins from passing through the intestinal mucosal barrier. Bile acids have the ability to attach to endotoxins or break them down into innocuous molecules. Through the body's excretion mechanism, bile acids remove toxins from the body. The bile acids aid in the hepatopancreas' and livers large-scale release of thinner cells. The bile acids lessen the harm that heavy metals, mycotoxins, and other dangerous compounds do to the hepatopancreas.

#### **Control Soft Shell and Abnormality Moulting Phase**

Shrimps will experience poor conditions, such as soft shells and sluggish growth, during the atypical molting phase. These phenomena have mostly been caused by bacterial infections, malnutrition, and a



lack of calcium sources. A bacterial infection that causes a soft shell is typically accompanied by aberrant body color, turbidity in the muscles, red body, etc. A balanced diet is necessary for shrimp to gain a significant amount of energy prior to molting. The shrimp body is at its weakest during each molt. The pathogen can easily infect a shell. When added to feed on a regular basis, bile acids accelerate the process of synthesizing nourishment and shield shrimp bodies from issues like soft shells, irregular molting, and other shell-related issues. External as well as internal factors have an impact on the molting processes of shrimp. Following their molting process, shrimp undergo tissue growth and absorbent enlargement. This is a significant volume-growth phase that is being followed by an organizational-growth process. The primary endogenous elements that affect shrimp molting times are the level of molting hormone (MIH). Shrimp can create molten ketone using the cholesterol in their diet. The sole source of cholesterol that the shrimp can synthesize is feed. Therefore, the use of bile acids has become a crucial tool to boost the frequency of molts in shrimp in order to speed up growth and metamorphosis. Shrimp growth can be greatly aided by the bile acids.

### Conclusion

In aquaculture production, feed is the primary source of nutrient loading, and its effects are critical to sustainable development, whether intensive or semi-intensive. This will increase the predictability of environmental effects and lessen the negative effects. Feeding and nutrition will be crucial to the continued advancement of shrimp growing techniques. Regardless of the culture system in which they are raised, shrimp and

other aquatic creatures depend primarily on an adequate supply of nutrients, both in terms of quantity and quality, for growth, health, and reproduction. Input supply (feeds, feed additives, etc.) must be guaranteed in order to meet the nutrient and energy needs of the species being cultivated as well as the system's production objectives. Exogenous bile acid supplementation can significantly increase the digestion and utilization of fat in shrimp diets by giving the fat additional energy. Bile acids have been shown to enhance growth performance, decrease feed costs, maintain feed quality, decrease hepatic fat accumulation, avoid fatty liver, bind to endotoxins, remove endotoxins from the body, and safeguard the health of the intestines and liver.

Feed additives called "bile acid" improve profitability, feed efficiency, water/pond quality, pellet quality, and health outcomes, all of which are important for sustainable aquaculture. Additionally, bile acid feed additives provide profitable efficient mycotoxin risk control and Sustainable shrimp production.

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