Role of Amino Acids in Stress Management and Growth in Aquatic Species

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Abstract

Amino acids (AAs) are critical for the growth and stress management of aquatic species, being the building blocks for protein synthesis and contributing to key physiological processes such as metabolism, immune response, and cellular signaling. The optimal balance of essential and non-essential amino acids in aquaculture diets enhances growth, reduces nitrogen wastes, and minimizes production costs. Climate change and environmental stressors, such as variable temperatures and low oxygen, severely challenge aquatic life, with dietary strategies that enhance resilience and stress tolerance being highly required. AAs influence stress responses and growth in fish and crustaceans, focusing on their role in protein synthesis, antioxidant defenses, and immune system enhancement. Specifically, certain AAs, including tryptophan, leucine, and arginine, play roles in stress amelioration and health promotion. The mTOR signaling pathway aids in protein synthesis regulation and muscle growth, indicating a critical role for AAs in growth performance. Balanced AAs in aqua feeds enhance growth performance and raise resilience to environmental stressors under sustainable aquaculture practices.

Keywords: Immune response, mTOR Signaling, Environmental stressors, Crustaceans

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Introduction

Recently, the nutritionist and food security state has been undermined due to climate change, pandemics, downturns of economics, and conflicts to provide a safe and healthy diet (FAO, 2022). Stable and reliable quality protein with a lower environmental footprint is mandatory for sustainable aquaculture development. An ideal amino acid profile that meets the protein requirements is an effective way to optimize growth and reduce cost and nitrogen waste in aquatic practices. AAs are used as building blocks for protein synthesis and promote growth. Furthermore, they play a crucial role in regulating feed intake, metabolism, immune response, cell signaling, and the health of farmed animals, including fish and crustaceans (Wu et al., 2014).

Climate change especially rising water temperatures is challenging for marine life. Long-term high temperatures will affect the food web, biomass development, and prospective yield of freshwater and marine aquatic species (Mugwanya et al., 2022). Therefore, advancement to gain climate resilience will be overcome by promoting the capacity of animals to adapt themselves according to climate changes. Understanding these changes that promote the aquatic animal's capacity to survive the environmental changes could have a significant effect on fish growth and resilience (Abisha et al., 2022; Lindmark et al., 2022).

The objectives of this chapter determine the role of amino acids in aquatic species, examine the impact of environmental stressors, and the mechanism of stress management, and promote sustainable aquaculture practices.

2. Significance of AAs in the Aquaculture Sector

The aquaculture sector makes a substantial contribution to human growth, development, and health while playing an increasingly important role in fulfilling the global demand for high-quality animal protein. In terms of food production, cultured fish and shellfish have surpassed wild-capture fisheries. Notably, formulated feeds account for more than 70% of total aquaculture production costs. A prominent concern regarding aquaculture is its ecological viability, as numerous aquaculture species exhibit substantial dietary protein and fishmeal requirements. Proteins and AAs, the fundamental elements of tissue synthesis, constitute the most expensive nutrients in animal husbandry, making them central to the creation of cost-effective and sustainable aquafeeds. Research highlights that a balanced supply of both EAAs and NEAAs in aquaculture nutrition significantly enhances growth, physiological development, stress resilience, and harvest efficiency, including processes like larval metamorphosis (Xinyu et al., 2021).

3. Amino Acids

AAs are an important class of biomolecules that have both carboxylate groups ($-COO^-$) and amino groups ($-NH_3^+$). Usually, the term 'amino acids' is known as α-amino acids, because both the amino and carboxyl groups are attached to the α-carbon as shown in Figure 1. Nevertheless, other types of AAs are present in nature, for instance, the β-amino acids, in which the amino and carboxyl groups are attached to different carbons in the backbone (Maloy, 2013).

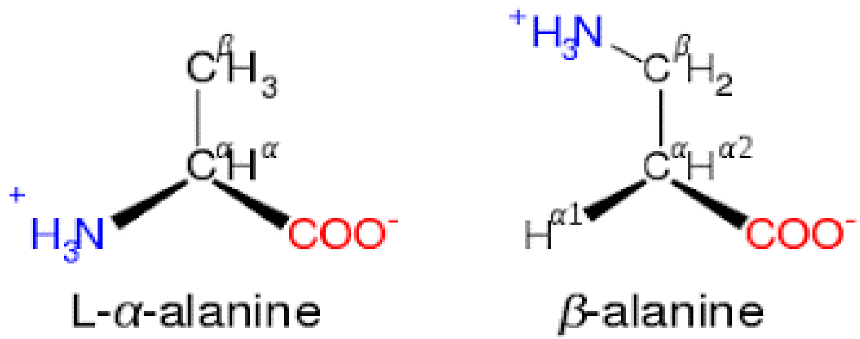

Fig. 1: Amino acid types (Forlano et al., 2023)

4. Amino Acid Classification Based on Nutrition

AAs are building blocks of protein that are essential for life. Helpful for the energy, breakdown of food particles, growth, tissue repair, and other body functions.

AAs are classified into three groups: essential AAs, non-essential AAs, and conditionally essential AAs as shown in Table 1. Essential AAs cannot be synthesized by the body and must come from a diet for protein synthesis. In contrast, non-essential AAs can be produced by the body and therefore do not need to be taken through diet. Conditionally essential AAs are usually not essential, except in times of illness and stress (Dietzen & Willrich, 2023).

Table 1: Essential, conditionally essential, and nonessential amino acids (Castellanos et al., 2006).

Essential	Conditionally Essential	Non-Essential
Histidine	Arginine	Asparagine
Leucine	Glutamine	Aspartic acid
Isoleucine	Tyrosine	Glutamic acid
Lysine	Cysteine	Serine
Methionine	Proline	Alanine
Phenylalanine	Glycine	
Threonine		
Tryptophan		
Valine		

5. Stress Response

The study of stress in aquatic species gains significant attention because it is closely connected to animal welfare. Since it is established that poor welfare circumstances reduce growth and survival, among other factors, fish farmers have begun to identify stress factors (Read, 2008). The use of functional foods, which have numerous beneficial impacts on the body beyond just nutrition, has emerged as a novel way to enhance overall health, reduce or modify the stress response, and ultimately enhance welfare (Cabanillas-Gamez et al., 2018; Olmos-Soto, 2005).

The initial stress response is based on the hormonal pathway, it is initially identified as a general adaptative syndrome (GAS), which triggers the other responses to stressors (Schreck & Tort, 2016).

6. Type of Stressors

There are two types of stressors in aquaculture which are the following.

6.1 Abiotic Stressors

Abiotic stressors are environmental factors that can cause stress to living organisms, such as chemicals, salinity change, osmosis, and temperature changes as shown in Fig. 2 (Bal et al., 2021). They can lead to physiological effects that disrupt homeostasis and affect cellular metabolism. These stressors can include various responses at molecular, cellular, and population levels, impacting ecosystem dynamics as shown in Figure 2 (Sulmon et al., 2015). Aquaculture and culture-based fisheries are increasingly affected by abiotic and biotic stressors, with abiotic stress referring to adverse environmental conditions that negatively influence organismal performance. The frequency of extreme weather events, exacerbated by both natural processes and anthropogenic activities, has intensified these challenges. Environmental stressors such as temperature fluctuations, flooding, droughts, and variability in precipitation patterns, salinity shifts, and oxygen depletion pose serious risks to aquatic ecosystems and aquaculture operations (Brander 2007; De Silva & Soto, 2009). Moreover, large-scale climatic changes, including sea-level rise, disruptions in ocean circulation, declining ocean productivity, and altered hydrological regimes, adversely affect aquatic species, fishing communities, and the sustainability of aquaculture (Anyanwu et al., 2015).

6.2 Biotic Stressor in Aquaculture

Biotic stressors are living organism activities, industrial waste, anthropogenic activity that cause stress, and acidification of water; leading to diseases, such as bacteria, fungi, viruses, etc (Tayeb et al., 2019) as shown in Figure 3 (Menon et al., 2023). Aquaculture practices face many stressors, like transport, crowding, grading, and vaccination. These stressors control the fish growth performance under the aquaculture system (Afonso, 2020). Under stressful conditions, fish try to overcome these stressors by changing their energy budget from normal metabolic processes such as growth, and survival and making changes in their physiological processes in response to stress conditions (Petitjean et al., 2019). AAs were used in growth as well as other processes such as for stress control (Aragao et al., 2008).

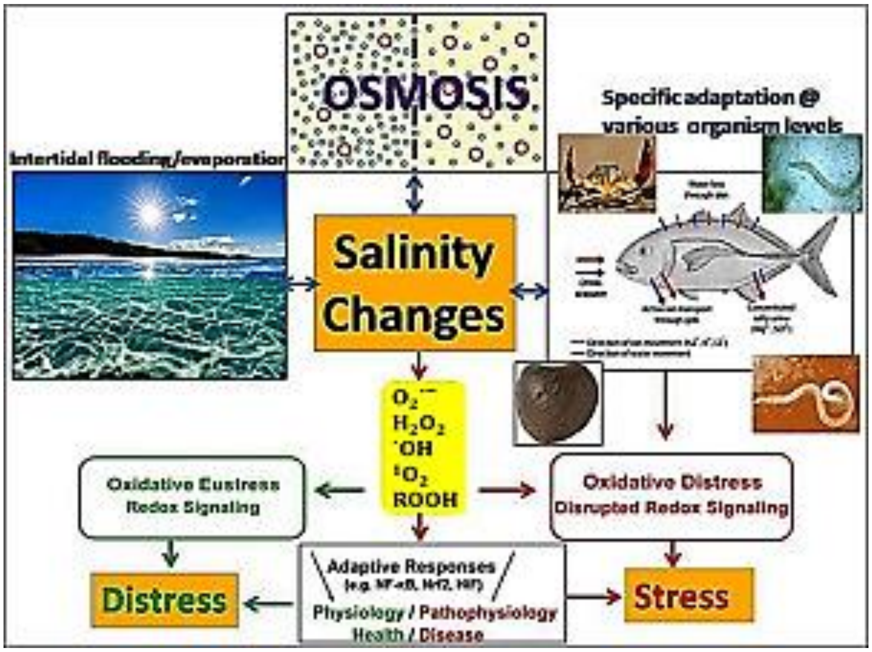

Fig. 2: Effect of abiotic stressors in aquaculture (Bal et al., 2021)

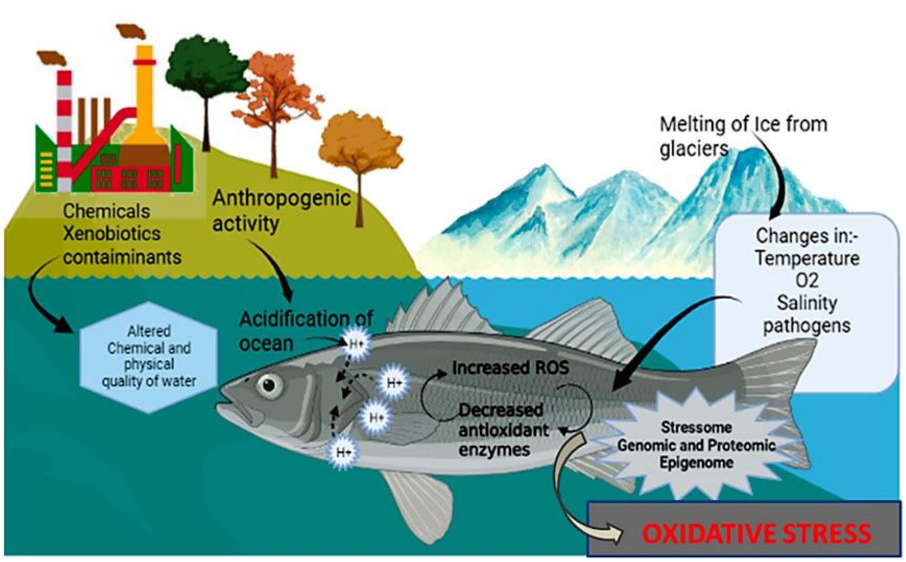

Fig. 3: Effect of abiotic stressors in aquaculture (Menon et al., 2023)

7. Physiological Role of Dietary Amino Acids under Fish Stress

Amino acid metabolism is directly affected by stressful conditions. During these situations, the requirements of the AAs are increased. Hence, AAs serve as both the parts of proteins as well as important biochemical substances (Conceicao et al., 2012).

For instance, branched-chain AAs (leucine, isoleucine, and valine) have a crucial role in protein synthesis regulation in skeleton muscles (Yoshizawa, 2004). An increased proteolysis activity is increased under stressful situations in fish and decreases the plasma levels of branched-chain AAs. Consequently, dietary administration of branched-chain AAs, particularly leucine seems to be a promising source to reduce stress in fish (Vijayan et al., 1997). According to some research, fish under stress have increased plasma content of total AAs and significantly lower concentrations of some free AAs. Alteration in plasma-free amino acid levels also indicates the requirements of free AAs in fish (Aragao et al., 2008).

Tryptophan (Trp) is an essential amino acid that reduces the stress response. It can be converted into two forms; one is serotonin and the other is melatonin. However, tryptophan is directly or indirectly involved in many physiological pathways. Dietary tryptophan is also a nutritional strategy to improve fish health (Lepage et al., 2003).

Higher vertebrates use arginine as a precursor to synthesize polyamines and nitric oxide. Fish-activated macrophages have been shown to produce nitric oxide (NO), which is crucial for cellular defense systems in fish. Furthermore, arginine also accelerates innate immune mechanisms and increases disease resistance against pathogen challenge (Costas et al., 2011).

Methionine is also an essential amino acid due to its significant role in the antioxidant defense system and immune response and it's also a precursor of cysteine formation, which is essential for taurine and glutathione formation. Methionine metabolism can be steered to three routes with health implications: (i) It serves as s-adenosylmethionine, which is subsequently decarboxylated and transformed into an amino propane donor that promotes polyamine turnover; (ii) s-adenosylmethionine immediately involved the methylation of multiple cell components, including DNA, adrenergic, dopaminergic, and serotonergic molecules; and (iii) it starts the mechanism, which forms glutathione from homocysteine (Grimble & Grimble, 1998; Li et al., 2007; Wu et al., 2004). Tyrosine is an intermediate for hormones and neurotransmitters, including epinephrine, norepinephrine, thyroxine, dopamine, triiodothyronine, and melanin. These play a vital role under stressful circumstances. Subsequently, tyrosine increases pigmentation development, growth, survival rate, feed intake, and immunity. During stress reactions, tyrosine is important, as reports show plasma-free tyrosine concentrations rise during acute stress responses (Costas et al., 2011).

8. Functions of Dietary Amino AAs in Fish Stress

Anti-oxidative defenses such as free radicals, while beneficial in specific physiological and evolutionary contexts, can become harmful when produced in excess, leading to oxidative damage to proteins, lipids, and DNA. This oxidative stress can result in cellular injury, dysfunction, and death (Fang et al., 2002). AAs contribute substantially to antioxidative defenses, functioning as precursors for the synthesis of antioxidant enzymes and scavengers of reactive oxygen species (ROS). Arginine, citrulline, glycine, proline, 4-hydroxyproline, taurine, and histidine are among the AAs recognized for their antioxidative properties as shown in Table 2 (Wu et al., 2013).

Dietary arginine supplementation has been shown to elevate the expression of antioxidative enzymes, such as Cu/Zn-SOD, peroxidase of glutathione, and catalase, thereby enhancing oxidative defenses in snout bream juvenile (Liang et al., 2018). Similarly, optimal levels of dietary methionine mitigate tissue peroxidative damage by increasing the activities of SOD and glutathione peroxidase in juvenile yellow catfish (Elmada et al., 2016). Additionally, dietary L-carnitine has demonstrated the ability to augment enzymatic antioxidant activity, including superoxide dismutase (SOD), catalase, and glutathione S-transferase (GST), in sea bream (Ma et al., 2008). According to Hu et al., (2014) synthesis of glutathione depends on glutamate, cysteine, and glycine which participate in tissue repairing, proliferation, and migration of enterocytes under oxidative conditions.

Table 2: Effect of amino acids on different fish species

Amino acids	Fish species	Functions in different fish species	References
Arginine	Channel catfish (*Ictalurus punctatus*)	Increase in the resistance of channel catfish to infection by E. ictalurid	Buentello and Gatlin (2001)
	Yellow catfish (*Pelteobagrus fulvidrac*)	Increases in lysozyme activities, as well as the phagocytic index and the respiratory burst of head–kidney leucocytes	Zhou et al., (2015)
	Nile tilapia (*Oreochromis niloticus*)	Increases in the metabolites, as well as total synthase and lysozyme activities in plasma	Yue et al., (2015)
Glutamine	Hybrid striped bass (*Morone chrysops × M. saxatilis*)	Increases in the production of superoxide by neutrophils; higher serum lysozyme activity	Cheng et al., (2012)
Methionine	European seabass (*Dicentrarchus labrax*)	Increases in peripheral leucocyte responses, complement activity, and bactericidal capacity; cellular and bactericidal activities in plasm	(Machado et al., 2015)
Lysine	Cobia (*Rachycentron canadum*)	Increase in blood leukocyte number	Zhou et al., (2007)
Leucine	Black carp (*Mylopharyngodon Piceus*)	Increases in mRNA levels or enzyme activities of immune defense effectors and in non-specific immunities; decrease in oxidative stress	Wu et al., 2017

9. Amino Acids and Stress Regulation in Crustaceans

Adequate dietary intake is essential for maintaining the resilience and health of cultured aquatic organisms as well as for attaining optimal growth (Pohlenz & Gatlin, 2014). AAs serve key functions in the immune systems of fish, crustaceans, and mammals (Li et al., 2007). Innate immune systems are used only to fight off infections in crustaceans because they lack an adaptive immune system (Vazquez et al., 2009). The prophenol-oxidase system is the main component of the immune response, stimulated by derivatives of microbial molecules. These components are not only phagocytic in nature but also release bioactive molecules to strengthen immune defense (Soderhall & Cerenius 1992). In addition to hemocytes, crustaceans possess a range of plasma proteins and humoral factors, including lectins, alpha-macroglobulin (for clotting), -binding proteins (lipopolysaccharide) β-glucan-binding proteins, peptides, and lysosome enzymes (Trichet, 2010; Vázquez et al., 2009). Certain AAs enhance immune responses and stress tolerance in crustaceans. Arginine, as a precursor for nitric oxide (NO), supports oxygenation in tissues and immunity (Wu et al., 2009). Tryptophan, a precursor for serotonin, has been shown to increase serotonin levels in the hemolymph of mud crabs, reducing aggressive behaviors and alleviating stress (Laranja et al., 2010). Tryptophan supplementation in Chinese crabs promotes limb regeneration, probably by modifying gene expression linked to regeneration and hepatopancreatic food digestion, which may be related to increased melatonin levels and the binding of serotonin and dopamine to their respective receptors and reducing stress (Zhang et al., 2019).

9.1 Mechanisms of Oxidative Stress Mitigation

Highly reactive molecules known as reactive oxygen species (ROS) can worsen oxidative stress brought on by radiation in animals, including chromosome abnormalities, protein oxidation, and muscle damage, as well as metabolic and morphologic alterations like increased muscle proteolysis and central nervous system dysfunction. Endogenous antioxidant systems play a crucial role in regulating ROS production and minimizing cellular damage. Key components of these defense systems include superoxide dismutase (SOD), glutathione, glutathione peroxidase, glutathione reductase, and catalase (Fang et al., 2002). Evaluating the impact of dietary AAs on antioxidative responses under diverse environmental conditions is imperative for elucidating their roles in stress mitigation.

10. Functions of Dietary AAs in Fish Growth

Protein is an essential element of all biological tissues and undergoes continual turnover involving synthesis and breakdown. In addition to being the essential building blocks of proteins, AAs are also vital for preserving homeostasis throughout the body (Wu, 2018). Currently, the National Research Council (NRC, 2011) provides dietary recommendations for essential amino acids (EAAs) in fish but does not specify requirements for non-essential amino acids (NEAAs) such as glutamate, glutamine, glycine, and proline. In a study by Jia et al., (2019), mortality rates were seen in young hybrid-striped bass that were given a refined diet free of glutamine and glutamate. Fish on a 60% fishmeal diet had a mortality rate of 97% by day 35 of the trial, whereas those on a completely purified diet that contained all AAs had an 89% mortality rate, and those on purified diets that lacked either glutamate or glutamine had a 39% mortality rate. These findings suggest that the endogenous synthesis of glutamate and glutamine is inadequate to support the growth and survival of hybrid-striped bass, underscoring the nutritional essentiality of these NEAAs for this species.

Protein synthesis usually outpaces protein breakdown in developing fish, leading to net protein accumulation (NRC, 2011). Intracellular protein synthesis relies on both an appropriate supply of AAs and energy. The growth performance or Protein deposition and growth performance seen in fish fed with various levels of AAs determine its optimum level of AA. Interestingly, one of the most important indicators of body weight gain in developing fish is protein deposition (Dumas et al., 2007).

10.1 Mechanisms of Fish Growth

Fish exhibit indeterminate growth, characterized by a continual increase in size throughout their lifespan, although their specific growth rate (%/day) diminishes with age. Muscle development in adult myotomal tissue is driven by both hyperplasia (an increase in muscle fiber number) and hypertrophy (an enlargement of muscle fiber size) (Johnston, 2001). Myogenesis, the process of skeletal muscle development, is regulated by myogenic regulatory factors (MRFs), which govern key processes like muscle progenitor cell specification, activation, and differentiation. Maintaining the integrity of muscle fibers requires a careful balance between protein synthesis and breakdown (Fuentes et al., 2013). A crucial component of cellular physiology, the mechanistic target of the rapamycin (mTOR) signaling pathway mainly aids in the start of protein synthesis (Wang & Proud, 2006). This pathway can be activated by intracellular AAs which involve mediators like Rheb, MAP4K3, and HVps34, and (Duan et al., 2015). Leucine, glycine, glutamine, and arginine are among the dietary AAs that are essential regulators of the mTOR pathway in fish (Chen et al., 2015).

11. Role of Amino acids in the Growth of Crustaceans

Crustaceans, which include prawns and crabs, are the second most popular aquaculture species worldwide and are a great source of protein for human use. Understanding the digestion, metabolism, and absorption of dietary protein, including AAs and tiny peptides, is crucial for the supply of sustainable and reasonably priced aquafeeds. The midgut gland known as the hepatopancreas is the main point for the absorption of peptides, AAs as well as proteins into the hemolymph (Li et al., 2021).

Moreover, AAs are precursors for the formation of lipids, glucose and less molecular weight (e.g., polyamines, histamine, hormones, and nitric oxide) have significant values, including the physical barrier, immune system, and antioxidant defenses. Consequently, both EAA and NEAA are required for the growth, molting, and development of crustaceans. There are some challenges in the use of AAs for research purposes because damage of AAs during the feed dispensation. Nowadays, a significant amount of our knowledge on the metabolism of AAs in crustaceans comes from research on fish and mammal species. The foundation of balanced and bioavailable AAs in the feed of crustaceans, as well as controlling the metabolic diseases for optimal growth and health status depends on basic research in this area as shown in Table 3 (Li et al., 2021).

Table 3: Effect of amino acids in crustaceans

Amino acids	Functions in Crustaceans	References
Arginine	Improves antioxidant and immune systems in shrimps *Fenneropenaeus chinensis* and *Marsupenaeus japonicus*	Jiang et al., (2006)
Glutamine	Improves wound healing, and maintenance of normal crustacean connective tissues in crustaceans.	Caskey et al., (2009)
Lysine	Improves immune functions, antioxidant defense systems, and energy metabolism in white-leg shrimp (*Litopenaeus vannamei*) and narrow-clawed crayfish (*Astacus leptodactylus leptodactylus* Eschscholtz, 1823)	Zhou et al., (2017); Safari et al., (2015)
Cysteine	Improves growth, antioxidant system, and stress resistance in white-leg shrimp (*Litopenaeus vannamei*)	Xia et al., (2018)
Glycine	Improves growth, antioxidant, and immune system in white leg shrimp (*Litopenaeus vannamei*)	Xie et al., (2014)
Tryptophan	Regulator of growth, reproductive function, and agonistic behavior in the black tiger shrimp (*Penaeus monodon*) and the mud crab (*Scylla serrata*)	Wongprasert et al., (2006); Laranjia et al., (2010)

AAs are very important in aquaculture species such as crustaceans. The balanced diet of crustaceans must contain essential AAs; all these AAs cannot be synthesized by the eukaryotic body (NRC, 2011). These are also known as the limiting AAs because these are directly involved in the growth, survival, and development of the animals. A deficiency of one essential amino acid results in limiting the intracellular synthesis of protein from AAs. For example, less protein retention rate due to the shortage of lysine (Xie et al., 2012). The supplementation of glycine in the diet of Pacific white shrimp increased the weight gain and specific growth rate (Xie et al., 2014). Other aspects stimulate the dietary requirements of AAs, depending on the stocking density, water quality, feeding rate, and feed formulation (Facanha et al., 2016; Zhang et al., 2018). Increasing the dietary arginine content (1.73-3.63%) improved the feed intake, growth, survival, and disease in the carps (Qi et al., 2019).

11.1 Mechanism

AAs increase growth by either providing building blocks for protein formation or accelerating the other signaling pathways (Wolfe, 2017; Wu, 2013). The mechanistic target of rapamycin (mTOR), an evolutionarily conserved protein kinase, is the main regulator of intracellular protein degradation through autophagy, cytoskeleton remodeling, and protein synthesis (Wu, 2013). AAs, like arginine, tryptophan, leucine, valine, and glutamine initiate the production of proteins in the gut and skeletal muscles by activating the mTOR signaling pathway which ultimately increases the growth performance (Li et al., 2011; Wu, 2018). The signaling pathway in crustacean species mTOR regulates growth, cell differentiation, metabolism, and molting (Abuhagr et al., 2014; Shyamal et al., 2018). Leucine and arginine administered intraperitoneally stimulate the mTOR signaling pathway and increase the expression of fish-TOR in the Chinese white shrimp (*Fenneropenaeus chinensis*; Sun et al., 2015).

Conclusion

Innovative strategies are needed to cope with constraints in resources and environmental stressors for sustainable aquaculture. AAs are important in promoting growth, managing the effects of stress, and immune defence in aquatic species. They enhance protein synthesis, and oxidative defence system, and modulate growth through pathways such as mTOR signalling. This chapter highlights the importance of balanced dietary AA profiles and physiological responses to stress, therefore making aquaculture sustainable while reducing dependency on conventional protein sources. Amino acid supplementation, especially with EAAs, can promote adaptive response to stress factors and enhance fish growth. Detailing the mechanisms underlying AA functions, it provides a framework for advancing aquaculture productivity. Future research should focus on improving AA supplementation to species-specific needs and increase adaptation to environmental challenges for resilient aquaculture systems.

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