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Review Article

Nutritional Significance of Amino Acids on Enhancing Production and Health Status of Tilapia Species

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Abstract

Aquaculture production of finfish has seen rapid growth in production volume and economic impact in the world. By 2030, aquaculture is expected to be the main source of fish, providing 50% of all fish consumed globally now. This is primarily due to consumer demand and furthermore to the diminishing wild catch fisheries. Tilapia is one of the most important fish species cultured more than 120 countries and territories around the world and contributes more than 10% of worldwide fish production. A balanced feed for fish must include satisfactory amount of all essential amino acids, fats, minerals and vitamins. Amino acids are essential for normal growth, reproduction, health and sustainable maintenance of fish metabolism. Due to high protein content, great amino acid profile, high nutrient digestibility, and absence of antinutrients, fish meal is a crucial protein source utilised to create nourishing fish feed. Replacing fishmeal is a major challenge in growing aquaculture industry to produce cost efficient feeds. Plant based ingredients are regarded as economical and nutritious protein source to replace fishmeal in aquafeed. However, concentrations of the essential amino acids are generally limited in plant based protein sources than in fish meal. Hence, providing well-balanced dietary essential amino acids in aquafeed is essential to regulate growth, health, reproduction and survival of fish.

Keywords: Amino Acids; Tilapia; Growth Performances; Health Status; Fish Meal; Requirement

Introduction

Global tilapia production

Significant advancements in tilapia farming have occurred around the world over the past three decades. Since the tilapia business is still expanding and becoming more commercialized, it has been dubbed the most significant aquaculture species of the twenty-first century and is currently the second most important farmed fish in the world, after carps [1].

Tilapia are popularly known as “aquatic chicken” and cultured in more than 100 countries in the world [2]. The second most common farmed fish nowadays, right behind carp is tilapia. Global production of farmed tilapia exceeded 5 million MT in 2016, and witnessed an average annual growth rate of 10% during 2010 - 2016 [3]. Tilapia production from aquaculture accounted for 10% of total finfish produced globally from farming in 2016. Among different tilapia species, Nile tilapia (*Oreochromis niloticus*) is the predominant one, and contributes to 75% of total farmed tilapia. The rest are contributed by hybrid red tilapia *O. niloticus* x *O. mossambicus* (15%), mozambique tilapia, *O. mossambicus* (5%), blue tilapia, *O. aureus* (2-3%) and other species (2-3%).

Genetically improved farmed tilapia (GIFT)

In Asia, South America, and Africa, tilapia is a crucial fish species for domestic markets. Tilapia's main destination market for export is the United States of America [3]. As a result, numerous selective breeding programmes for Nile tilapia have been launched, including a significant non-commercial breeding operation by World Fish Centre that produced the genetically improved farmed tilapia (GIFT). Many nations have received the GIFT strain. The GIFT strain was the basis population for 10 out of 17 Nile tilapia breeding initiatives [4].

Important breakthroughs that fueled the rapid growth of tilapia industry include production of mono-sex male population and genetically improved fast growing strain through selective breeding program. The GIFT (genetically improved farmed tilapia) strain of Nile tilapia is an emerging selective bred fish from the base population involving 8 different strains of Nile tilapia, *Oreochromis niloticus.* A quicker-growing strain of Nile tilapia that was appropriate for both small-scale and commercial aquaculture was the goal of the Genetic Improvement of Farmed Tilapia project, which was started by the World Fish Centre. The Nile tilapia was chosen due to its acceptance in aquaculture, brief generation period of about 6 months, excellent natural tolerance to varying water quality, good disease resistance, and flexibility in adapting to various farming systems [5]. The GIFT programme increased the growth rate of *O. niloticus* by 85% overall and by 12-17% on average over the course of five generations. The desirable traits of this genetically enhanced strain include high yielding, excellent breeding, efficient conversion of organic and agricultural wastes into high quality protein, resistance to diseases, extreme hardiness, tolerance to overcrowding conditions, and the ability to grow in both fresh and brackish water.

Production of GIFT tilapia with respect to nutrition and feeding

Significant improvements have been made over the years in the feed (nutrition, feed processing) and feeding management of tilapia, which triggered the intensification of tilapia farming. However, the industry is facing significant challenges with rising feed cost which constitutes the major operational cost of tilapia production. The cost of diet is largely accounted for dietary protein, hence efforts to find new protein sources that are more affordable have been ongoing. Studies have demonstrated that, with the use of supplemental amino acids, fish meal can be effectively replaced by plant and alternative animal protein sources, while protein utilization can also be improved [6,7].

Current fish meal level in tilapia feed is not more than 5% in main tilapia producing countries. Nevertheless, effective use of alternative protein sources and further improvements in protein utilization require more precise understandings on the amino acid requirements of tilapia. Besides, Nile tilapia has been genetically improved over 15 generations of selection with 10% improvement in weight gain per generation [8]. This makes it essential to regularly validate and update the nutrient requirement data of tilapia in order to meet their new genetic demands. Replacing fishmeal is a major challenge in growing aquaculture industry to produce cost efficient feeds [3,8]. Plant based ingredients are regarded as economical and nutritious protein source to replace fishmeal in aquafeed. However, concentrations of the essential amino acids are generally limited in plant based protein sources than in fish meal. Hence, providing well-balanced dietary essential amino acids (EAA) in aquafeed is a major approach to regulate growth, health, reproduction and survival of fish [9].

Use of soybean meal and corn gluten meal in fish diets

Compared to other plant protein sources, soybean meal is the most usual in diets for different aquaculture species due to its ready market availability, high nutritional value, low cost, high protein content and good amino acid profile, but it is deficient in sulphur-amino acids and contains endogenous antinutrients [10]. This feedstuff is generally included at 20-50% of total formulation in fish diets.

A significant by-product of maize wet milling, corn gluten meal (CGM) contains a high protein content (minimum 60%), a high degree of digestibility (> 90%), and adequate levels of methionine, leucine, and glutamic acid [11,12], however it lacks lysine, arginine, and tryptophan [13]. Moreover, CGM is a rich source of carotenoids, mainly xanthophylls. This product is a good alternative for fish meal, as a source of dietary protein for freshwater, marine water, warm water and cold water fishes. There have been successful efforts to incorporate corn gluten meal in aquatic diets and decrease the amount of fish meal consumed by fish [14-17]. These studies have shown that 20 to 60% of CGM could be used instead of fish meal.

Amino acids

Protein is composed of amino acids. On the basis of dietary needs for growth, amino acids are classified as nutritionally essential (indispensable) and nonessential (dispensable) for fish. Essential amino acids are those that animals cannot or cannot produce enough of on their own to meet their needs. A diet must contain conditionally necessary amino acids when rates of use are higher than rates of synthesis. Aquatic animals are capable of producing all non-essential amino acids in sufficient amounts. Specific amino acids may be added to the diet to: (1) improve the nutritional value and chemo attractiveness of aquafeeds with little fishmeal inclusion; (2) maximise metabolic efficiency variation in juvenile and subadult fishes; (3) reducing cannibalism and aggressive behaviours; (4) boosting larval performance and survival; (5) regulating the timing and effectiveness of spawning; (6) enhancing fillet flavour and texture and (7) increasing resistance and tolerance to environmental stresses. The production of balanced aquafeeds using functional amino acids has enormous promise for increasing the productivity and profitability of aquaculture around the world [9].

Chemical synthesis or fermentation are the two methods used to create amino acids. Racemic mixes of the D- and L-forms of the amino acids are formed as the end products of chemical production. All amino acids are now supplied through fermentation in modern commercial activity, with the exception of those containing sulphur (Methionine). This is because only the L-isomeric form, which may be used by animal cells, is produced during fermentation. Chemical synthesis is used to develop DL-methionine sources because the D-isomer is also useful for methionine sources.

Supplementation of crystalline amino acids

Essential amino acids can be regulated in aquafeeds by combining protein sources with various amino acid profiles or by adding crystalline amino acids. Crystalline amino acids are utilised commercially as economical supplements to make up for specific amino acid inadequacies in terrestrial animals. Dietary amino acid supplementation resulted in increased whole-body protein and decreased whole body fat in fish species [12]. Aquatic feeds have traditionally used fish meal as their preferred source of protein, but since world stocks have stopped growing, it is becoming less common and more expensive. As a result, the aquaculture feed industry became more and more acclimated to using less expensive proteins derived from processed plant proteins, residues from farming and fishing, or even meat from terrestrial production animals. These alternate components might have a crude protein (CP) concentration similar to fish meal, but they might also be less digestible and lacking in one or more of the 10 necessary amino acids (EAAs). Due to this, newer formulation techniques that consider nutrient availability have to be used, particularly with regard to EAAs. For a variety of fish and shrimp species, EAAs needs have been determined. In rendered plant and animal by-products, methionine and lysine are the first two limiting amino acids. By merely raising the dietary inclusion levels of the feedstuffs that include entire sources of the targeted EAAs, formulating for EAAs can result in over-formulated feeds with excessive amounts of CP and other nutrients. Supplementing the diet with crystalline amino acids is a more valid approach. Understanding the amount of digestible EAAs present in the ingredients that can be used in the formula is a first step in applying a nutrient-based formulation technique. It is valued highly to formulate using CP, EAA, and energy digestibility in accordance with the current formulation techniques used by the majority of existing feed firms [18]. To balance the amino acids in fish diets, the optimum protein concept should be utilized [19,20]. A study found that, crystalline- amino acid may replace 19% of dietary protein without negatively affecting growth performance or feed efficiency in juvenile turbot [21].

Amino acid requirement of tilapia

IAA requirements for Nile tilapia *Oreochromis niloticus* growth were initially established by Santiago and Lovell [21], with values published for lysine (1.43%), arginine (1.18%), and glycine (1.09%), methionine (0.75%), histidine (0.48%), isoleucine (0.87%), leucine (0.95%), valine (0.78%), phenylalanine (1.05%), threonine (1.05%), tryptophan (0.28%). Several studies [22-25] have since been carried out demonstrating a significant difference in the ideal amounts of IAAs for Nile tilapia.

Lysine

Lysine is commonly one of the most constrained amino acids in ingredients used to make commercial fish feeds, especially when

Amino acid	Model used	Response variable	Requirement
Lysine	Broken line	WG	1.43
	Broken line	WG	2.32
	Linear	WG / FCR	1.8
	Quadratic	WG	1.56
	Nonlinear	N deposition	1.63 & 2.09
	Linear	N deposition	1.56
	Broken line	WG	1.31
	Quadratic	Fillet yield	1.46
Methionine	Broken line	WG	0.75
	Broken line	WG	0.85
	Quadratic	WG	1.57
	Quadratic	WG	0.91
	Broken line	WG	0.49
	Quadratic	WG	0.99
	Nonlinear	N deposition	0.73 & 0.94
	Linear	N deposition	0.99
	Quadratic	WG	1.13
Threonine	Broken line	WG	0.75
	Nonlinear	N deposition	0.83 & 1.05
	Quadratic	WG	1.33
	Linear	N deposition	1.45
	Quadratic	WG	1.20
Tryptophan	Broken line	WG	0.28
	Linear	N deposition	0.37
Arginine	Broken line	WG	1.18
	Quadratic	WG	1.36
	Linear	N deposition	1.95
Isoleucine	Broken line	WG	0.87
	Linear	N deposition	0.88
Leucine	Broken line	WG	0.95
	Quadratic	WG	1.25
	Linear	N deposition	1.5
Valine	Broken line	WG	0.78
	Linear	N deposition	1.18
Histidine	Broken line	WG	0.48
	Linear	N deposition	0.54
	Quadratic	WG	0.82
Phenylalanine	Broken line	WG	1.05
	Linear	N deposition	1.57

Table 1: EAA requirements (% of diet) of Nile tilapia published in various studies.

fishmeal is substituted with plant protein sources [26]. Lysine supplementation in fish diets not only improves fish growth but also enhances the production of fillets [27]. As a result, the amount of lysine in the diet has a significant impact on the health and growth of fish. With the availability of feed grade lysine on the market, its addition to diets based on plant proteins facilitates the decrease of dietary crude protein in an efficient manner without impacting fish development performance [26]. This dietary approach can also reduce fish's excretion of soluble phosphorus and ammonia [28]. Also, dietary lysine supplementation is beneficial for increasing immunological responses and gastrointestinal development in agastric fish (Jian carp) [29].

Lysine metabolism in fish

Carnitine, a substance made from lysine, is necessary for transporting long-chain fatty acids from the cytosol to the mitochondria for oxidation. Supplementing with dietary carnitine may help promote development, guard against the toxicity of ammonia and xenobiotics, improve tolerance to sudden temperature changes and the stress they cause, and improve reproductive efficiency [30].

Lysine requirement of tilapia species

First study on the lysine requirement of tilapia was documented that fingerling stage Mozambique tilapia (*Sarotherodon mossambicus*) require 1.62% lysine in the diet [31]. Later another study reported even lower dietary lysine requirements (1.43%) for Nile tilapia fry [21]. After a long interval, another study investigated lysine requirement for fingerling tilapia. The study found an optimal dietary lysine requirement of 2.32% on total basis and 2.17% on digestible basis for maximizing weight gain of fingerling Nile tilapia [32]. These numbers are significantly greater than the ones found for tilapia in previous studies. A study showed a linear increase in the weight gain of fingerling Nile tilapia in response to increasing lysine levels (1.05-1.80%), suggesting the optimal requirement to be 1.80% of diet [25]. In another study, using quadratic model, it was determined that the requirement was estimated to be only 1.56% of diet for optimizing weight gain of fingerling Nile tilapia [24].

Tryptophan

Tryptophan is an essential amino acid and a precursor of several compounds, including serotonin (5-hydroxytryptamine), an important neurotransmitter that affects physiologic functions and behavioral responses of fish [12]. Dietary tryptophan is being successfully supplemented in diets to suppress aggression and cannibalism in fish species [33]. Dietary tryptophan supplementation also improves growth performance of fish, and its deficiency may increase the incidence of scoliosis, lordosis and eye cataracts in salmonids [34,35].

Tryptophan metabolism in fish

Serotonin (5-hydroxytryptamine), a neurotransmitter, and melatonin, an antioxidant, can both be derived from tryptophan [36]. Under conditions of intensive rearing, aggressive behaviors and cannibalism of carnivore fish may result in significant output losses. Chronic increases in serotonin levels and metabolism in the brain are linked to decreased aggression. Inhibiting aggression in young rainbow trout [33], lowering cannibalism and stress-induced anorexia in young groupers [37], and preventing a stress-induced cortisol surge are all possible effects of dietary supplementation with L-tryptophan [38]. Dietary supplementation of L-tryptophan at a minimum level of 1.36% reduced the high density group stress and improved growth performance in *Cirrhynus mrigala* [39]. Use of tryptophan may be a potential nutritional method for health management in aquaculture since persistent cortisol elevation negatively impacts growth, feed intake, protein accretion, immunity, and disease challenge [40] (e.g., transport, handling, and vaccination).

Tryptophan requirement for tilapia

The tryptophan requirement for various fish species has been reported to range from 0.1 to 0.6% of diet [12]. Previous studies have estimated the dietary tryptophan requirement values of Nile tilapia such as 0.37% of diet [22], 0.34% of diet [41], 0.31% of diet [42], 0.29% of diet [43] and 0.28% of diet [21].

Arginine

Arginine is an essential amino acid and the major substrate for the production of nitric oxide and plays important roles in the modulation of the immune response [44], improving growth performance [45] and lowering fish environmental stress [46]. Because it is plentiful in protein (as a peptide-bound AA) and tissue fluid (as phosphoarginine, a key ATP reservoir), fish have relatively high dietary arginine requirements. Additionally, de novo synthesis of arginine is either scarce or nonexistent in fish. Arginine is involved in several cellular metabolic pathways, including the urea cycle and synthesis of creatine, nitric oxide (NO) and polyamines.

Arginine metabolism in fish

Numerous physiological mechanisms directly or through its derivatives involve arginine. This amino acid serves as the most prevalent nitrogen carrier for tissue proteins and is utilised in a variety of biosynthetic processes that involve important regulatory enzymes including arginase, nitric oxide synthase, and arginyl-tRNA synthetase, among others [47]. Thus, arginine exhibits exceptional metabolic and modulatory plasticity in animal cells, including those of fish [48], and acts as a precursor for the synthesis of creatine, ornithine, proline, glutamate, polyamines, and nitric oxide [45,49]. Effects of arginine on both the innate and adaptive immune response have been demonstrated in fish, where arginine may act through NO to combat pathogens, through polyamines, directly by affecting gene expression or by regulating nutrient availability for immune cells by endocrine control [50].

Arginine requirement of tilapia

Many studies have estimated the dietary arginine requirement of Nile tilapia to be 1.18% [21], 1.36% [51], 1.95% [22] and 1.82% of diet [52].

Methionine

Methionine is commonly the first limiting amino acid in many fish diets, especially those with high quantities of plant protein sources including soybean meal, peanut meal, and copra meal [53]. Understanding the role of sulphur amino acids in fish nutrition is crucial for replacing fishmeal with plant-based feedstuffs, which is a common replacement approach. Because plant foods are methionine-limited, certain fish species frequently experience development retardation, decreased feed efficiency, and lenticular cataracts as a result of methionine insufficiency [54,55].

Chemical techniques are used to create commercial amounts of methionine and its derivatives. The DL-form of methionine is easily obtainable. The natural isomer of methionine, l-methionine, is easily absorbed and effectively utilised by mammals. D-methionine oxidase is required for transaminating D-methionine into a keto-acid, and transaminases are required for converting the ketoacid into L-methionine [56]. Cystine can replace approximately 70% of the dietary requirement for methionine in mammals [57].

Methionine metabolism in fish

Cysteine is synthesized from methionine so that it can be absorbed into proteins. Methionine is used to generate the dispensable amino acid cysteine [55]. As a result, it should be suitably provided in the diet. Its presence in the diet at the necessary quantity may allow the body to use less methionine than would otherwise be needed for its synthesis [55]. Methionine is a precursor of several substrates, including proteins, phospholipids, biogenic amines, carnitine, cysteine, choline, polyamines, and other metabolic intermediates. These substrates play a variety of physiological roles in cell division and development. Methionine content in the diet has been shown to have an impact on fish growth performance, feed intake, and carcass quality in both excess and limited conditions [31,58,59].

Methionine requirement for tilapia

Methionine requirement of tilapia was first investigated long back in 1980s. A study showed that fingerling stage Mozambique tilapia require 0.53% methionine (0.74% cysteine) in the diet [31]. Fry Nile tilapia's nutritional methionine later needs to be reported as 0.75%, when test diets supplied a constant cysteine level of 0.15% [21]. Recently, few studies have reported requirements for sulfur amino acids for juvenile Nile tilapia such as 0.49% methionine [60] and 0.85% methionine + cysteine (M+C) [61], 0.91% methionine (0.10% cysteine) and 0.99% M+C [22].

Amino acid	Product	Function	Species
Arginine	Nitric oxide	Facilitate neurological function and development	Tilapia
	Nitric oxide	Kill invaded microorganisms	Channel Catfish
	Nitric oxide	Increase disease resistance during external stress	Senegalese sole
	Nitric oxide	Regulate vascular tone, blood flow, osmolarity in gill and cell signaling	Killifish
	Polyamines	Spermine induce intestinal maturation	Sea bass

Alanine, Glutamic, Serine	Directly	Appetite	Many fishes
Arginine and Methionine	Spermine	Induce larval intestinal maturation	Seabass
Arginine, Methionine and Glycine	Creatine	High energy storage, antioxidant	Arctic charr
Histidine	Directly and carnosine	Protection against pH change	Salmon
Leucine	Hydroxyl- β-methyl-butyrate	Immunity modulation, Cell signaling	Various fishes
Lysine, Methionine	Carnitine	Lipid transporter on mitochondrial membrane	Various fishes
Methionine	Choline	Structure in membrane, neurotransmitter, betaine synthesis	Various fishes
Proline	Hydroxyproline	Enhance growth, Collagen function	Salmon
Phenylalanine, Tyrosine	Triiodothyronin, thyroxine	Influence metamorphosis	Sole
	Triiodothyronin, thyroxine	Enhance growth performance	Channel catfish
	Triiodothyronin, thyroxine	Influence pigmentation	Japanese flounder
	Melanin	Influence pigmentation	Rainbow trout
	Epinephrine, norepinephrine	Neurotransmitters that modulate stress responses	Flounder
Tryptophan	Serotonin	Modulate cortisol release, behavior and feeding	Rainbow trout
	Melatonin	Improve testicular development	Masu salmon
Taurine	Directly	Osmotic pressure regulation	Carp
	Directly	Hardness adaptation	Channel catfish
	Directly	Gut development	Cobia
	Directly	Retinal development	Glass eel

Table 2: Roles of amino acids in physiological functions and metabolism of aquatic animals.

Conclusion

The development of AA-balanced feeds can counteract the environmental effects on aquaculture animals, enhance growth performance, and increase the profitability of the aquaculture sector. There is convincing evidence that many fish species can be dietary supplemented with L-lysine HCl, DL-methionine, threonine, and tryptophan to make up for their inadequacies in plant feedstuffs. Additionally, generating feed-grade arginine and glutamine on a large scale holds a lot of potential for maintaining growth and health in aquaculture. As AA nutrition technology is used to create functional and environmentally conscious aquafeeds, they will continue to progress over the next ten years. Based on their critical functions in cell metabolism and physiology, we assume that dietary supplementation with specific amino acids may be advantageous for: (1) enhancing the chemo-attractive property and nutritional value of aquafeeds with low fishmeal inclusion; (2) maximising efficiency of metabolic transformation in juvenile and subadult fishes; (3) suppressing aggressive behaviors and cannibalism; (4) increasing larval performance and survival; (5) mediating the effects of dietary supplementation with specific amino acids. As an outcome, including amino acids into feed composition will guarantee increased shrimp and fish production in a successful direction.

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