

New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds

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Abstract Recent evidence shows that some amino acids and their metabolites are important regulators of key metabolic pathways that are necessary for maintenance, growth, feed intake, nutrient utilization, immunity, behavior, larval metamorphosis, reproduction, as well as resistance to environmental stressors and pathogenic organisms in various fishes. Therefore, conventional definitions on essential and nonessential amino acids for fish are challenged by numerous discoveries that taurine, glutamine, glycine, proline and hydroxyproline promote growth, development, and health of aquatic animals. On the basis of their crucial roles in cell metabolism and physiology, we anticipate that dietary supplementation with specific amino acids may be beneficial for: (1) increasing the chemo-attractive property and nutritional value of aquafeeds with low fishmeal inclusion; (2) optimizing efficiency of metabolic transformation in juvenile and sub-adult fishes; (3) suppressing aggressive behaviors and cannibalism; (4) increasing larval performance and survival; (5) mediating timing and efficiency of spawning; (6) improving fillet taste and texture; and (7) enhancing immunity and tolerance to environmental stresses. Functional amino acids hold great promise for development of

balanced aquafeeds to enhance the efficiency and profitability of global aquaculture production.

Keywords Amino acids · Fish · Health · Growth · Aquafeeds · Aquaculture

Abbreviations

AA	Amino acids
GABA	γ -Aminobutyrate
BCAA	Branched-chain amino acids
HMB	Hydroxyl- β -methyl-butyrate
NAC	<i>N</i> -Acetyl-cysteine
NO	Nitric oxide
NOS	Nitric oxide synthase
P5C	Pyrroline-5-carboxylate
T3	Triiodothyronine
T4	Thyroxine

Introduction

Amino acids (AA) are building blocks for protein. On the basis of needs from diet for growth, AA were traditionally classified as nutritionally essential (indispensable) or non-essential (indispensable) for fish (Table 1). Essential AA are those that either cannot be synthesized or are inadequately synthesized *de novo* by animals relative to needs. Conditionally essential AA must be provided from the diet under conditions where rates of utilization are greater than rates of synthesis. By definition, all nonessential AA can be synthesized adequately by aquatic animals.

Dietary protein is the major and most expensive component of formulated aquafeeds (Wilson et al. 2002). Fishmeal is generally considered to be the most ideal

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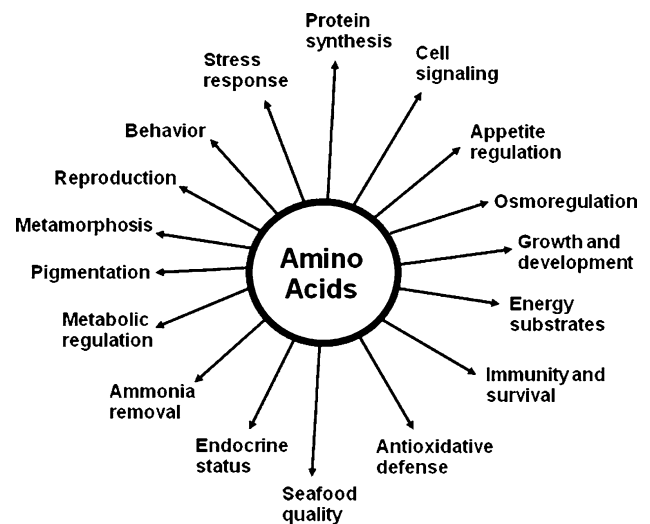
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Table 1 Nutritionally essential and nonessential amino acids for fish and other aquatic animals

Essential AA	Nonessential AA	Conditionally essential AA
Arginine	Alanine	Cysteine
Histidine	Asparagine	Glutamine
Isoleucine	Aspartate	Hydroxyproline
Leucine	Glutamate	Proline
Lysine	Glycine	Taurine
Methionine	Serine	
Phenylalanine	Tyrosine	
Threonine		
Tryptophan		
Valine		

protein source for aquatic animals, despite its static global production, seasonal/geographical variability in quality and composition, and concern as a vector of contamination (Trushenski et al. 2006). Over the past decades, there have been rapid increases in global aquaculture and demand, as well as price and competition for this valuable feedstuff. With a growing interest in non-fishmeal protein sources for aquafeeds, it is important to sustain comparable levels of feed intake, feed conversion efficiency, as well as growth rate and survival in fishes. Therefore, establishment of optimal dietary requirements of AA and characterization of alternative protein/AA sources have been a major focus of fish nutrition research.

Emerging evidence from studies with both aquatic and terrestrial animals shows that many AA regulate key metabolic pathways that are crucial to maintenance, growth, reproduction, and immune responses (Fig. 1). They are termed “functional AA”. Identification and dietary supplementation of those AA or their biologically active metabolites is expected to offset adverse effects of replacement of fishmeal from aquafeeds, therefore restoring food intake and growth. Of particular interest, the “least-cost” principle of aquafeed formulation has been evolving in recent years towards customer-based aquafeeds, which has led to the concepts of “functional aquafeeds” and “environmentally oriented aquafeeds”. Functional aquafeeds are defined as feeds supplemented with specific ingredient(s) to achieve desirable efficiency of metabolic transformation, growth performance, health, and/or compositional traits of aquacultured animals at various developmental stages. Environmentally oriented aquafeeds are defined as feeds modified to minimize negative impacts of environmental changes (including salinity, ammonia, extreme temperatures, and stressors imposed by husbandry handling) on growth, health, and reproduction of aquacultured animals. Both functional and environmentally oriented aquafeeds are designed to meet needs of the

**Fig. 1** Roles of amino acids in growth, development and health of fish

industry and consumers. Amino acids are potentially important ingredients for these two types of feeds. This review summarizes current knowledge on roles of AA in fish nutrition and proposes new strategies to guide development of AA-balanced aquafeeds.

Alanine, β -alanine, aspartate and asparagine

Alanine and aspartate are the major glucogenic precursors and important energy substrates for fish. In addition, aspartate is essential to purine nucleotide synthesis in all cell types. Moreover, alanine is a preferred carrier of nitrogen for inter-organ AA metabolism in fish (Mommensen et al. 1980). Aspartate and asparagine represent ~10% of AA in plant and animal proteins, but dietary aspartate may be extensively catabolized by the small intestine of fish, as established for mammals. Thus, most of the circulating aspartate is derived from synthesis by skeletal muscle (Wu 1998). It is noteworthy that aspartate aminotransferase is up-regulated in gill, white and red muscles (Bystriansky et al. 2007), and liver (Aas-Hansen et al. 2005) during salinity acclimation or migration.

Alanine can stimulate feeding response of certain fishes although the underlying mechanisms are unknown (Shamushaki et al. 2007). Interestingly, dietary supplementation with 5% pyruvate can reduce nitrogen excretion into the surrounding water via enhancement of alanine formation, without a negative effect on the growth and vitellogenin synthesis in Atlantic salmon (Olin et al. 1992). At present, there is a little information regarding supplementation of aspartate or asparagine to fish diets. Due to rapid oxidation and a lack of toxicity, alanine and aspartate are often used to balance nitrogen in fish nutrition research.

β -Alanine (a product of aspartate decarboxylation) is a constituent of carnosine (β -alanyl-L-histidine), anserine (β -alanyl-1-methyl-L-histidine), carcine (β -alanyl-histamine), and balenine (β -alanyl-3-methyl-L-histidine). Carnosine and anserine are antioxidants and important buffers in skeletal muscle of aquatic animals, especially migratory pelagic marine fishes (Snyder et al. 2008). Elevated levels of β -alanine in plasma or tissue in response to acute handling stress or an environmental change have been reported for fish (Aragão et al. 2008) and oysters (Powell et al. 1982); however, the physiological significance of this finding has not been elucidated. Ogata (2002) found that dietary supplementation of β -alanine increased intramuscular concentration of carnosine, but not anserine, in yellowtail. Kim et al. (2003) observed no positive effect of β -alanine supplementation on growth performance of Japanese flounders. Carnosine is abundant in fish but absent from plants. Supplementing carnosine to all-plant protein-based diets did not appear to enhance growth or feed efficiency in rainbow trout (Snyder et al. 2008); however, potential use of carnosine for fish under stressful conditions remains to be determined.

Arginine, citrulline and ornithine

Fish have particularly high requirements for dietary arginine, because (1) it is abundant in protein (as a peptide-bound AA) and tissue fluid (as phosphoarginine, a major reservoir of ATP); and (2) its de novo synthesis is limited or even completely absent. Elasmobranchs and ureogenic teleosts may be able to convert citrulline into arginine via argininosuccinate synthase and lyase in liver (Mommsen et al. 2001). However, it is unknown whether there is net synthesis of citrulline or arginine by this organ in aquatic animals. There is circumstantial evidence for an arginine-sparing effect of dietary glutamate in channel catfish (Buentello and Gatlin 2000), but this effect may result from an inhibition of arginine degradation. At present, pathways for arginine synthesis from glutamine, glutamate, proline, or AA other than citrulline have not been established for fishes.

Arginine is one of the most versatile AA by serving as the precursor for the synthesis of protein, nitric oxide (NO), urea, polyamines, proline, glutamate, creatine and agmatine in terrestrial animals (Wu and Morris 1998). Arginine also plays a crucial role in regulating endocrine and reproductive functions, as well as extra-endocrine signaling pathways (including AMP-activated protein kinase and the target of rapamycin) (Jobgen et al. 2006; Yao et al. 2008). Fish can produce NO by tetrahydrobiopterin-dependent NO synthase (NOS; Buentello and Gatlin 1999), as well as urea plus ornithine by arginase (Gouillou-Coustans et al. 2002). The resultant ornithine can be used for the synthesis of

proline or polyamines. A substantial decrease in plasma concentrations of arginine and ornithine has been observed for Senegalese sole subjected to acute stress (Aragão et al. 2008).

Except for endothelial NOS (eNOS), genes for inducible NOS (iNOS) and neuronal NOS (nNOS) have been cloned for various fish (Bordieri et al. 2005). While physiological levels of NO play an important role in cellular signaling, a high concentration of NO synthesized by iNOS can result in AA and protein modification, oxidative stress, apoptosis, and killing of target cells (Galli 2007; Mannick 2007). Inducible NO production in macrophages is affected by extracellular arginine availability or nutrition of channel catfish (Buentello and Gatlin 1999). nNOS is expressed in the brain and gill of fish that regulates neurological function and development, vascular tone and osmotic pressure (Hyndman et al. 2006). In addition, arginine can stimulate the release of various hormones such as insulin, growth hormone, and glucagon. Indeed, arginine is a more potent activator of insulin release than glucose in fish (Mommsen et al. 2001). Growth- and health-promoting effect of dietary arginine beyond meeting requirement for protein synthesis has been reported for some fishes. For example, Buentello and Gatlin (2001) found that the survival of channel catfish in response to challenge with *Edwardsiella ictaluri* critically depended upon dietary arginine levels. Despite extensive work with mammalian species (Wu and Morris 1998), little is known about the efficacy of citrulline to replace arginine in fish diets.

Branched-chain AA (BCAA)

Leucine, isoleucine plus valine account for 18–20% AA in plant and animal proteins. As an activator of the target of rapamycin (a protein kinase), leucine is considered as a functional AA to stimulate muscle protein synthesis and inhibit proteolysis in mammals (Nakashima et al. 2007). However, such a role for leucine has not been investigated for aquatic animals. Choo et al. (1991) reported that excessive leucine increased feed efficiency but depressed food intake, growth, and protein deposition of rainbow trout likely due to antagonism among AA (particularly BCAA). Notably, in recent years, there has been growing interest in the stimulatory effect of supplemental β -hydroxyl- β -methyl-butyrate (HMB; a leucine metabolite) on growth and immunity in several coldwater aquacultured fishes (Siwicki et al. 2003). However, such a benefit has not been observed in warm water species, including tilapia and hybrid striped bass (Li and Gatlin 2007). This discrepancy may be explained by species differences in leucine metabolism among fishes. In addition, studies with fishes (e.g., rainbow trout, Arctic charr, and even shrimp) have revealed that BCAA can be rapidly mobilized from white muscle and

red muscle during exhaustive exercise (Milligan 1997) or sea water acclimation (Bystriansky et al. 2007), but the rates of leucine release vary greatly among species.

Glutamate, glutamine, and γ -aminobutyrate (GABA)

Glutamine is one of the most abundant free α -AA in fish plasma and muscle, whereas glutamate and its decarboxylation product (GABA) are neurotransmitters present at high concentrations in the brain. In addition, GABA is an inducer of metamorphosis in abalone (Morse et al. 1979). Moreover, glutamine is essential for the synthesis of purine and pyrimidine nucleotides in all cells. Through renal ammoniogenesis, glutamine also plays an important role in regulating the acid–base balance in the body. Further, glutamine stimulate muscle protein synthesis in mammals (Wu et al. 2007), but such information is not yet available for fish. As a major energy substrate for leukocytes and a key modulator of cytokine and NO production, glutamine is crucial to the immune response in fish (Buentello and Gatlin 1999; Li et al. 2007).

Glutamate and glutamine represent $\sim 20\%$ of AA in plant and animal proteins, but they could be completely degraded by the gut of aquatic animals, as reported for terrestrial mammals (Wu 1998). Thus, most of glutamate and glutamine in plasma may be synthesized from BCAA and α -ketoglutarate in skeletal muscle. Glutamate is a substrate for glutamine synthesis by ATP-dependent glutamine synthetase, whereas glutamine is hydrolyzed by phosphate-dependent glutaminase to generate glutamate (Anderson et al. 2002). This intracellular and intercellular glutamine–glutamate cycle is well known for mammals, but has not been firmly established for fishes. As major enzymes initiating glutamate degradation, glutamate dehydrogenase is expressed in fish liver and white muscle, whereas glutamate transaminase is abundant in all fish tissues (Wilson 2002). Glutamine synthetase is abundant in brain, intestine, liver, red muscle, gill/kidney, white muscle, and heart of fish, and the hepatic protein is up-regulated by cortisol (Vijayan et al. 1996) or high environmental ammonia (Anderson et al. 2002). Both glutamate and glutamine are important energy substrates in fish, but tissue-specific metabolism of the two AA is fully defined for aquatic animals.

High dietary levels of GABA inhibits food intake by Japanese flounder (Kim et al. 2003). In contrast, studies with Atlantic salmon indicate that dietary supplementation with 5% α -ketoglutarate can reduce nitrogen excretion into the water without a negative effect on growth and vitellogenin synthesis (Olin et al. 1992). Importantly, dietary glutamine supplementation enhances weight gain, feed intake, gain:feed ratio, intestinal development, and digestive enzyme activities in Jian carp (Lin and Zhou 2006). With the recent commercial availability of commercial mixtures of

feed-grade glutamine and glutamate (e.g., AminoGutTM, Ajinomoto Inc., <http://www.Ajinomoto.com>), it will be of practical importance to determine whether this new product can enhance growth performance and immunity in fish (including shrimp).

Glycine and serine

Glycine and serine are interconverted primarily in liver and kidneys by tetrahydrofolate-dependent hydroxymethyltransferase. These two AA participate in gluconeogenesis, sulfur AA metabolism, one-carbon unit metabolism, and fat digestion (Fang et al. 2002), and also stimulate feed intake by many fishes (Shamushaki et al. 2007). Glycine may function to regulate gene expression in fish, as dietary supplementation with glycine has been shown to increase hepatic thyroxine 5' monodeiodinase activity in rainbow trout (Riley et al. 1996), therefore enhancing the efficiency of nutrient absorption and anabolic events. Interestingly, glycine has a critical role in the osmoregulatory responses of fishes and shellfishes (e.g., oysters) to environmental stress. For example, oysters rapidly take up free glycine from the surrounding water, and they synthesize gill glycine in response to rapid changes in salinity or anoxia (Powell et al. 1982). Importantly, glycine-enrichment can enhance the survival of oysters after being transferred to freshwater from seawater (Takeuchi 2007) and may be an innovative method to improve oyster larviculture. Similarly, tissue levels of serine in aquatic animals (including shrimp) are increased by abrupt salinity change (Silvia et al. 2002) or hardness change (Buentello and Gatlin 2002). At present, little is known about whether dietary serine supplementation may optimize fish survival and growth in suboptimal culture conditions.

Histidine

Histidine is an abundant AA in plasma albumin of fish (Szebedinszky and Gilmour 2002). It is also rich in fish muscle as a free AA or carnosine. Histidine participates in one-carbon unit metabolism, therefore affecting DNA and protein synthesis. In addition to serving as an energy fuel during starvation, histidine is a major component of non-carbonate buffers protecting fish against changes in pH resulting from hypoxia, burst-swimming, and increased lactacidosis. The capacity of the non-carbonate buffer system among various fish species varies considerably, which is likely attributable to long-term adaptation to environments. Interestingly, intramuscular histidine concentration is substantially increased before spawning migration of salmon (Mommsen et al. 1980). It is possible that histidine metabolism and its dietary requirement in fish are regulated by many environmental and endocrine factors.

Because histidine and its related imidazole derivatives (anserine and carnosine) confer desirable taste and texture (e.g., sweetness, heaviness and thickness), dietary supplementation of histidine can improve sensory attributes (e.g., flavor) of aquacultured seafoods (Ogata 2002). Additionally, Førde-Skjærvik et al. (2006) reported that dietary supplementation of histidine increased intramuscular histidine levels and pH, while reducing muscle gapping in Atlantic cod post-mortem. This, in turn, contributes to a higher quality fillet.

Lysine, carnitine and cadaverine

Lysine is often one of the most limiting AA in ingredients used for production of commercial fish feeds, especially when fishmeal is replaced by plant protein sources (Mai et al. 2006a). Therefore, dietary lysine levels critically affect fish growth performance and health. With the commercial availability of feed-grade lysine, its addition to plant protein-based diets allows for cost-effective reduction of dietary crude protein without affecting fish growth performance (Mai et al. 2006a). This nutritional strategy can also decrease the excretion of ammonia and soluble phosphorus from fish (Cheng et al. 2003). In addition, dietary lysine supplementation is effective in enhancement of immune responses and gastrointestinal development of agastric fish (Jian carp) (Zhou 2005).

Lysine is a substrate for the synthesis of carnitine, which is required for the transport of long chain fatty acids from the cytosol into mitochondria for oxidation. Potential benefits of dietary carnitine supplementation include growth promotion, protection against the toxicity of ammonia and xenobiotics, improved acclimation to extreme temperature changes and associated stress, and enhanced reproduction performance (Harpaz 2005). However, because of differences in experimental conditions, data on the nutritional value and potential economical return of carnitine supplementation have not been consistent for various types of fishes and even within the same species. Further research is necessary to explain this discrepancy and identify an effective dose of carnitine and responsive conditions.

Cadaverine is a decarboxylation product of lysine during putrefaction of animal tissue, such as fishmeal staling. Tapia-Salazar et al. (2004) reported that combined supplementation of cadaverine and histamine can enhance feed consumption and growth of blue shrimp, rather than being responsible for the reduced nutritional value of stale fishmeal as suggested by Cowey and Cho (1992). Histamine can regulate blood flow and intestinal development in mammals (Ou et al. 2007), but these effects have not been investigated for aquatic animals.

Phenylalanine and tyrosine

Phenylalanine can be converted to tyrosine by tetrahydrobiopterin-dependent phenylalanine hydroxylase in liver and kidneys. Thus, adding tyrosine to diets for fish can reduce requirement for phenylalanine. Research on supplementing phenylalanine and tyrosine to aquafeeds and their potential influences on aquatic animals is currently limited. Tyrosine is a common precursor for important hormones and neurotransmitters, including thyroxine (T4), triiodothyronine, epinephrine, norepinephrine, dopamine, and melanin. These molecules have important regulatory roles (Chang et al. 2007; Yoo et al. 2000); hence, dietary levels of phenylalanine and tyrosine could profoundly influence pigmentation development, feed intake, growth performance, immunity, and survival of fish in natural environment. Consequently, dietary requirements for phenylalanine and tyrosine of fish increase substantially during metamorphosis (Pinto et al. 2008). Additionally, oral administration of T4 to carp, channel catfish and flounder fish increased protein digestibility, digestive enzyme activity, nutrient retention, growth rate, and feed efficiency (Garg 2007).

Polyamines

Polyamines (putrescine, spermidine, and spermine) are naturally occurring polycationic substances that are essential for cell proliferation and differentiation. They are synthesized from arginine- or proline-derived ornithine in mammals (Wu et al. 2008); however, these pathways have not been established for fish. Spermine is relatively abundant in most fishes, possibly indicating active polyamine synthesis or limited degradation. Natural dietary sources of polyamines for fishes have not been fully defined. It has been postulated that gastrointestinal microbes in fish larvae can produce polyamines and facilitate development of the small intestine. Early study indicated that dietary supplementation of putrescine did not enhance growth performance of juvenile rainbow trout (Cowey and Cho 1992). However, supplementing spermine to microparticulate diets can increase digestive enzyme activity and promote intestinal maturation, therefore enhancing the survival of larval fish (e.g., European sea bass) (Péres et al. 1997). It should be noted that high doses of polyamines are toxic to aquatic animals and reduce growth performance (Cowey and Cho 1992).

Proline and hydroxyproline

Proline is traditionally thought to be a dispensable AA for fish and promotes feed intake. In mammals, proline is synthesized from arginine, ornithine, glutamine, and glutamate in a tissue-specific manner (Wu and Morris 1998).

However, pathways for proline synthesis in fish are not well defined. Indeed, whether proline can be sufficiently synthesized in fish to meet requirements for maintenance, maximal growth or optimal health remains largely unknown. Dabrowski et al. (2005) reported the presence of pyrroline-5-carboxylate (P5C) reductase in the liver and intestine of rainbow trout, but the source of P5C was not identified. These authors also found that proline concentration in muscle of rainbow trout alevin was dependent on dietary proline and that endogenous synthesis of proline from glutamate could not meet the requirement for proline (Zhang et al. 2006). Therefore, proline is now considered as a conditionally essential AA for fish in early life stage and perhaps also for adult fish.

Hydroxyproline content in fish meal is much higher than that in plants proteins. In animals, hydroxyproline is produced via the post-translational hydroxylation of proline in protein (primarily collagen) by vitamin C-dependent prolyl hydroxylase. Fish skin and bones are important sources of collagen. Female fish (e.g., ayu) experience considerable collagen demobilization and lose hydroxyproline during maturation and spawning (Toyohara et al. 1997). Hydroxyproline has been demonstrated as the only free AA in tissues that is positively correlated to the growth rate of juvenile salmon (Sunde et al. 2001). Hydroxyproline may also be a signaling molecule that regulates cellular redox state and apoptosis (Phang et al. 2008). Recently, Aksnes et al. (2008) found that dietary supplementation of hydroxyproline, but not proline, increased growth rate and modified bone composition of salmon. Although the underlying mechanisms are unknown, these findings support the notion that AA derivatives are potent growth promoters for fish. Addition of hydroxyproline to plant protein-based aquafeeds may be beneficial for maximizing survival and growth of aquatic animals.

Sulfur amino acids: methionine, cysteine and taurine

Methionine is usually the first limiting AA in many fish diets, especially those containing high levels of plant protein sources, such as soybean meal, peanut meal, and corpa meal (Mai et al. 2006b). Pathways of methionine transmethylation, remethylation, transsulfuration for the synthesis of cysteine and taurine are known for mammalian liver, and are likely present in fish despite possible quantitative differences among species. For example, the conversion of methionine into taurine has been reported for fish (Wilson 2002), but little is known about the intermediates, enzyme activities, as well as hormonal and nutritional regulation. Interestingly, cysteine dioxygenase, one of the two rate-controlling enzymes in taurine synthesis from cysteine, is up-regulated by dietary methionine at the transcriptional level, but not affected by dietary

taurine (Gaylord et al. 2007). Transcription of other enzyme, cysteine sulfinic acid decarboxylase, in the liver of rainbow trout is not influenced by dietary taurine, but inhibited by dietary methionine (Gaylord et al. 2007). It would be important to determine whether changes in maximum activities of enzymes measured at saturated concentrations of substrates translate into alterations in the flux of methionine to taurine. S-Adenosylmethionine, which is an important methyl group donor for creatine and spermidine synthesis (Grillo and Colombatto 2007), is crucial for metabolic regulation in fish. However, it is not known whether dietary supplementation with S-adenosylmethionine may enhance fish survival or growth.

Methionine and its derivatives are produced commercially by chemical processes. Methionine is commonly available in the DL-form. L-Methionine, the natural isomer, is absorbed readily and used efficiently by animals. D-Methionine must be transaminated into α -ketoacid by D-methionine oxidase, and the α -ketoacid is then converted into L-methionine by transaminases (Wu and Thompson 1989). In contrast to mammals, the utilization of methionine hydroxyl analog (a synthetic non-nitrogenous compound) by fishes or crustaceans is not highly efficient (Wilson 2002). Of particular interest, because of the presence of a high deacetylase activity in the cytosol of animal tissue, N-acetyl-methionine is an excellent precursor of sulfur AA in fish (Keembiyehetty and Gatlin 1995).

There is limited information about nutritional essentiality of cysteine for fish. It has been estimated that cysteine can spare 40–60% of methionine in the diets for various fishes (Wilson 2002). Dietary supplementation with N-acetyl-cysteine (NAC) is an effective means to augment the provision of cysteine, therefore enhancing the synthesis of glutathione (a major antioxidant tripeptide in cells) in animals (Wu et al. 2004). Some studies reveal that adding glutathione to diets can spare 75% requirement of methionine by hybrid striped bass (Keembiyehetty and Gatlin 1995), suggesting that glutathione synthesis is an important metabolic fate of methionine. Interestingly, dietary NAC can reduce cutaneous mucus viscosity, however, it failed to confer protection to rainbow trout or Atlantic salmon infection by *Paramoeba* spp. (amoebic gill disease) infection (Powell et al. 2007).

Taurine is not incorporated into proteins, but plays important roles in fat digestion, antioxidative defense, cellular osmoregulation, as well as development of visual, neural and muscular systems (Fang et al. 2002; Omura and Inagaki 2000). Taurine is abundant in fish meal and animal products (particularly marine invertebrate products), but absent from plants. Convincing evidence shows that supplementing taurine to all-plant protein diets can promote growth and efficiency of feed utilization by carnivore fish, such as rainbow trout (Gaylord et al. 2007) and Japanese

flounder (Kim et al. 2003), but not common carp (Kim et al. 2008), suggesting suboptimal de novo synthesis of taurine by certain species. Additionally, taurine concentration in tissues of certain fish is greatly influenced by changes in salinity (Dabrowski et al. 2005) or hardness (Buentello and Gatlin 2002). However, potential impacts of these environmental factors on sulfur AA metabolism, utilization and requirement are unknown. Nonetheless, dietary supplementation of taurine can promote intestinal development of cobia larvae, which may provide a new means to improve efficiency of larviculture (Salze et al. 2008).

Threonine

Most of the previous research on threonine was focused on minimum dietary requirements for various fishes, because of its deficiency in plant proteins. Dietary threonine levels can affect immunity in mammals (Li et al. 2007). In addition, threonine is the major component of mucin in the small intestine, therefore regulating intestinal barrier integrity and function. However, potential use of threonine in aquafeed beyond meeting the dietary requirement has received little attention.

Tryptophan, serotonin and melatonin

Tryptophan can be converted to serotonin (5-hydroxy-tryptamine; a neurotransmitter) and melatonin (an antioxidant) (Fang et al. 2002). Aggressive interactions and cannibalism of carnivore fish may cause substantial production losses under conditions of intensive rearing. Chronic increase in brain concentrations and turnover of serotonin are associated with suppressed aggression. Dietary supplementation with L-tryptophan can inhibit aggression in juvenile rainbow trout (Hseu et al. 2003), reduce cannibalism and stress-induced anorexia in juvenile grouper (Höglund et al. 2007), and prevent a stress-induced cortisol surge (Lepage et al. 2003). Because prolonged elevation of cortisol negatively affects growth, feed intake, protein accretion, immunity and disease challenge (Vijayan et al. 1996), use of tryptophan may be a promising nutritional strategy for health management in aquaculture (e.g., transport, handling, and vaccination).

Brain serotonin is synthesized in neurons, while peripheral serotonin is formed and released by gut mucous in response to different stimuli such as gastric distension, hyperosmotic solutions or presence of glucose. Both endogenous and exogenous (dietary) serotonin can inhibit food intake by fish (e.g., European sea bass) and terrestrial animals (Rubio et al. 2006). Melatonin is produced in the pineal organ, retina and gastrointestinal tract of fish, and is degraded in liver. The synthesis of melatonin in fish pineal organ is regulated by photoperiod and plays an important

role in mediating testicular maturation of certain fish, including masu salmon (Amano et al. 2004) and Indian carp (Bhattacharya et al. 2007). Exogenous administration of melatonin to fish can increase plasma concentrations and also stimulate testicular maturation. It should be borne in mind that the dose, timing, photoperiod and season are important factors that affect the efficacy of exogenous melatonin in mediating fish reproductive performance.

Challenges of uses of AA in aquafeeds

There are technical difficulties in the use of crystalline AA for aquafeeds. A primary concern is leaching of free AA into water environment, as well as their degradation by enterocytes and/or microflora in the gastrointestinal tract (Wu 1998). Second, differences in rates of intestinal absorption between synthetic and protein-bound AA may reduce the efficiency of crystalline AA (Murai et al. 1982). A wide variety of microbinding processes have been developed to ameliorate these problems. However, most of the available methods were either registered or patented, instead of being published in peer-reviewed journals. In addition, there may be species-specific response to the microencapsulation process. For example, certain binding with lipids causes low utilization of arginine by abalone, probably because of low lipase activity in the abalone gut and subsequent failure to degrade the fat coating arginine particles (Britz et al. 1997). Third, supplementing AA to aquafeeds (especially purified diets) may influence acid-base and electrolyte balances, ratios of AA in intestinal lumen and plasma, as well as digestion and absorption of nutrients. Fourth, the Maillard reaction in the process of feed extrusion can substantially reduce the bioavailability of free AA, especially lysine and arginine (Csapó et al. 2008). Fifth, pathways of AA metabolism and its regulation by neural, endocrine, and environmental factors in aquatic animals are poorly understood, therefore limiting development of growth-promoting means through targeted metabolic control. Sixth, research on technologies that allow efficient delivery of limiting AA or functional AA to fish larvae through live feeds is still limited (Saavedra et al. 2008). Overcoming those obstacles would result in a substantial improvement in utilization efficiency of supplemental AA for the formulation of aquafeeds.

Conclusion and perspectives

Amino acids play important and versatile roles in fish nutrition and metabolism. These functions include cell signaling (e.g., NO, arginine, glutamine, leucine, proline, and polyamines); appetite stimulation (e.g., alanine, glutamate,

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

Amino acid	Product	Function	Species	Reference
Amino acids	Various proteins	Structure, transport, regulation, immunity, signaling, and fuels	All animals	Li et al. (2007)
Ala, Glu and Ser	Directly	Appetite	Many fishes	Shamushaki et al. (2007)
Arg	NO	Kill invaded microorganisms	Channel Catfish	Buentello and Gatlin (1999)
Arg	NO	Facilitate neurological function and development	Tilapia	Bordieri et al. (2005)
Arg	NO	Regulate vascular tone, blood flow, osmolarity in gill, and cell signaling	Killifish	Hyndman et al. (2006)
Arg and Met	Spermine	Induce larval intestinal maturation	Sea bass	Péres et al. (1997)
Arg, Met, and Gly	Creatine	High energy storage; antioxidant	Arctic charr	Bystriansky et al. (2007)
Cys, Glu, and Gly	Glutathione	Antioxidant and cell signaling	All animals	Wu et al. (2004)
Glu and Gln	Directly	Ammonia removal	Rainbow trout	Anderson et al. (2002)
Glu	GABA	Promote metamorphosis	Abalone	Morse et al. (1979)
Glu	GABA	Regulate food intake	Japanese flounder	Kim et al. (2003)
Gln	Directly	Increase growth, feed efficiency and gut development	Carp	Lin and Zhou (2006)
Gln	Directly	Fuel for macrophage; Cell signaling	Channel catfish	Buentello and Gatlin (1999)
Gln, Gly, and Asp	Nucleotides	Genetic information storage and expression, biosynthesis, immunity and reproduction	Various fishes	Li and Gatlin (2006)
Gly	Directly	Increase hepatic T4 5' monodeiodinase	Rainbow trout	Riley et al. (1996)
Gly	Directly	Osmoregulation	Oyster	Takeuchi (2007)
His	Directly and carnosine	Protection against pH change	Salmon	Mommsen et al. (1980)
Leu	HMB	Immunity modulation; Cell signaling	Various fishes	Li and Gatlin (2007)
Lys and Met	Carnitine	Lipid transporter on mitochondrial membrane	Various fishes	Harpaz (2005)
Met	Choline	Structure in membrane; neurotransmitter; betaine synthesis	Various fishes	Mai et al. (2006b)
Proline	P5C	Redox regulation; Cell signaling	Possibly in fish	Phang et al. (2008)
Proline	Hydroxyproline	Enhance growth; Collagen function	Salmon	Aksnes et al. (2008)
Phe and Tyr	T4, T3	Influence metamorphosis	Sole	Pinto et al. (2008)
Phe and Tyr	T4, T3	Enhance growth performance	Channel catfish	Garg (2007)
Phe and Tyr	T4, T3	Influence pigmentation	Japanese flounder	Yoo et al. (2000)
	Melanin	Influence pigmentation	Rainbow trout	Boonanuntanasarn et al. (2004)
Phe and Tyr	Epinephrine, norepinephrine	Neurotransmitters that modulate stress responses	Flounder	Damasceno-Oliveira et al. (2007)
Phe and Tyr	Dopamine	Down-regulated immunity	Shrimp	Chang et al. (2007)
Trp	Serotonin	Modulate cortisol release, behavior and feeding	Rainbow trout	Lepage et al. (2003)
Trp	Melatonin	Improve testicular development	Masu salmon	Amano et al. (2004)
Taurine	Directly	Osmotic pressure regulation	Carp	Zhang et al. (2006)
Taurine	Directly	Hardness adaptation	Channel catfish	Buentello and Gatlin (2002)
Taurine	Directly	Gut development	Cobia	Salze et al. (2008)
Taurine	Directly	Retinal development	Glass eel	Omura and Inagaki (2000)

HMB hydroxyl- β -methyl-butyrate; NO nitric oxide; P5C pyrroline-5-carboxylate; T3 triiodothyronine; T4 thyroxine

proline, and serine); growth and development regulation (e.g., arginine, glutamine, hydroxyproline, leucine, and T4); energy utilization (e.g., NO, T4, and carnitine); immunity (e.g., NO, arginine, glutamine, and dopamine); osmoregulation (e.g., glycine, taurine, β -alanine, and arginine); ammonia detoxification (e.g., glutamate, glutamine, and citrulline); antioxidative defense (e.g., glutathione, cysteine, glutamine, glycine, and taurine); metamorphosis (e.g., tyrosine, T4, and GABA); pigmentation (e.g., T4 and melanin); gut development (e.g., taurine, glutamine, arginine, threonine, and polyamines); neuronal development (e.g., NO, arginine, taurine, and creatine); stress responses (e.g., tryptophan, serotonin, branched-chain AA and glutamine); reproduction (e.g., NO, polyamines, arginine, melatonin, and hydroxyproline); and suppression of aggressive behavior (e.g., tryptophan and serotonin) in aquatic animals (Table 2). In addition, certain AA (glutamate, histidine, and glycine) influence taste, texture, and even post-mortem seafood quality. Dietary supplementation of AA or derivatives, modulation of their metabolism, or a combination of the two may provide new strategies to develop AA-balanced feeds that can offset environmental impacts on aquacultured animals, improve growth performance, and profitability of the aquaculture industry. Compelling evidence shows that dietary supplementation of L-lysine HCl, DL-methionine, threonine, and tryptophan to compensate for their deficiencies in plant feedstuffs, is highly cost-effective for many fish species. Additionally, achieving large-scale feed-grade arginine and glutamine hold great promise for growth and health management in aquaculture. The coming decade will witness continuing advances in AA nutrition technologies and their application to formulate functional and environmentally oriented aquafeeds.

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