

# DIETARY REQUIREMENTS OF ARGININE FOR FARMED FISH SPECIES

## Abstract

Protein stands out as the most costly and crucial component within fish diets, providing amino acids (AA) essential for energy, growth, protein synthesis, and serving as substrates for key metabolic pathways. Beyond its role in protein synthesis, the term "functional AA" is employed to characterize amino acids involved in cellular processes. The deficiency or imbalance of these functional AAs can result in impaired body metabolism and homeostasis. In recent decades, amino acids have emerged as an unconventional and adaptable domain for enhancing disease resistance, immune response, reproduction, behavior, and more. Arginine, among the essential amino acids, is recognized as one of the functional amino acids in animals. The dietary requirement of arginine in fish spans between 3.0 and 8.1% of dietary protein. This amino acid is implicated in diverse cellular processes such as ureagenesis, immune function, antioxidant defense, somatotrophic axis, stress responses, and ammonia detoxification in fish. To delve deeper into the effects of arginine in fish, a book chapter focusing on arginine in various fish species has been published. It encompasses variations in arginine requirements among different fish species, the modulation of ureagenesis and ammonia detoxification, the interaction of arginine with lysine and glutamine, the modulation of insulin and growth hormone responses, cortisol response, modulation of the antioxidant system, immune responses, and disease resistance.

**Keywords:** Arginine, Dietary proteins, Amino Acids, farmed fish

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## I. INTRODUCTION

Amino acids (AA) constitute the fundamental building blocks of proteins, with a total of 20 amino acids required for proper bodily functions. Enzymatic breakdown of amino acids derived from protein-rich foods like meat, fish, dairy products, nuts, and beans occurs, resulting in their individual components. These building blocks are then reabsorbed and reconstructed as necessary for growth, maintenance, and the regulation of bodily processes. Two categories of amino acids exist: (a) Essential amino acids (EAA) and (b) Non-essential amino acids (NEAA). Eight amino acids are classified as essential because the body cannot synthesize them adequately and must obtain them from food, while the remaining twelve NEAA can be endogenously produced. Since the body cannot store amino acids, a continuous process of protein turnover or recycling takes place, necessitating a constant supply of proteins in the diet. Essential amino acids include leucine (Leu), isoleucine (Ile), threonine (Thr), tryptophan (Trp), phenylalanine (Phe), valine (Val), methionine (Met), and lysine (Lys). Amino acids like histidine (His) and arginine (Arg) are synthesized in tissues but not in sufficient amounts for normal growth, leading to their classification as semi-essential amino acids. In fish, arginine is considered an essential amino acid, while in humans, it is conditionally essential due to low activities of arginine biosynthetic enzymes in adult fish. Unlike in mammals, fish can convert glutamate, glutamine, and proline to citrulline, which is then converted to arginine in the kidneys. Fish primarily excrete ammonia through their gills, relying less on adenosine triphosphate (ATP) for the urea cycle. Arginine serves as a substrate for synthesizing biologically active metabolites in fish, including nitric oxide (NO), creatine, and polyamines. Dietary arginine elevation in fish increases serum insulin (INS) and insulin-like growth factor-I (IGF-I) levels, activates adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK), and stimulates the target of rapamycin (TOR) signaling pathway. Additionally, arginine regulates immune-modulatory functions in fish, impacting both innate and adaptive immune responses, inhibiting leukocyte apoptosis, and enhancing resistance against bacterial disease. Nevertheless, the effects of arginine vary based on dosage, fish growth and immune responses, health conditions, and environmental factors. Dietary arginine deficiency retards fish growth, while appropriate supplementation promotes growth and immune responses. Excessive arginine intake, however, inhibits both fish growth and immune responses.

## II. BIOSYNTHESIS OF ARGININE AT JUVENILE STAGE OF FISH

Arginine, also recognized as L-arginine (symbol Arg or R), is an  $\alpha$ -amino acid primarily utilized in the biosynthesis of proteins. Comprising an  $\alpha$ -amino group, an  $\alpha$ -carboxylic acid group, and a side chain featuring a 3-carbon aliphatic straight chain concluding with a guanidino group, arginine maintains a charged aliphatic amino acid status at physiological pH. In the early developmental phases of fish, arginine synthesis can occur through the urea cycle, owing to the heightened enzymatic activity within this cycle. The fish urea cycle initiates with the fixation of ammonia and glutamate into glutamine, catalyzed by the mitochondrial enzyme glutamine synthase (GS). In fish, the enzyme responsible for carbamoyl phosphate synthesis is CPS III, which derives its nitrogen source from glutamine rather than ammonia. While many teleosts exhibit ammoniotelic behavior in adulthood, they adopt a ureotelic state during early life stages, producing a significant quantity of urea. Zebrafish and Atlantic cod embryos are ureotelic, showcasing elevated activities of urea cycle enzymes. Rainbow trout, despite displaying heightened CPS III and ornithine

transcarboxylase (OTC) activities in early development stages, remain ammoniotelic during this phase, with at least 50% of nitrogenous waste being in the form of ammonia rather than urea.

- 1. Biosynthesis of Arginine at Adult Fish:** In adult teleosts, ammonia excretion predominantly occurs through the gills; nonetheless, the genes encoding most urea cycle enzymes are detectable, and some enzyme activities are observable in adult fish tissues. Tilapia exhibits higher activities of CPS III and other urea cycle enzymes in the muscle compared to the liver. Similarly, African catfish (*Clarias gariepinus*) predominantly localizes these enzymes in muscle tissues rather than the liver. In adult trout, the maximum CPS III activity is noted in muscle tissue, suggesting a limited biosynthetic capacity for endogenous carbamoyl phosphate and arginine in adult fish. Dietary incorporation of citrulline results in increased arginine levels in both muscle and blood plasma in rainbow trout, indicating potential promotion of arginine synthesis through citrulline supplementation. Additionally, the activities of urea cycle enzymes have been reported to significantly increase in certain fish species under specific conditions, such as crowding or high pH. Therefore, despite its low activity, arginine synthesis holds physiological significance. As fish lack the ability to endogenously synthesize arginine adequately for optimal growth, supplemental arginine in the fish diet becomes necessary.
- 2. Arginine Biosynthesis via Urea Cycle and Intestinal–Renal Axis:** The enzymes involved in the urea cycle include carbamoyl phosphate synthetase, ornithine transcarbamoylase, argininosuccinate synthetase, argininosuccinate lyase, and arginase. While ammonia is commonly regarded as the primary excretion product for nitrogenous waste, some fish possess ureagenesis enzymes and a functional urea cycle. This mechanism likely serves as a means of ammonia detoxification in certain fish species, wherein ammonia is converted to urea using bicarbonate and ATP. The conversion process involves the transformation of ammonia into carbamoyl phosphate, L-citrulline, arginine succinate, L-arginine, urea, and L-ornithine. L-ornithine is subsequently recycled back into the urea cycle. rephrase the following paragraph without changing its technical terms and meaning: "

In the mammalian liver, urea production primarily arises from the urea cycle rather than arginine biosynthesis due to the elevated activity of arginase in this organ. Consequently, the primary source of endogenous arginine production is renal arginine synthesis, constituting what is known as the intestinal–renal axis. In this axis, L-proline, L-glutamine, and L-glutamate undergo conversion to L-citrulline within the mitochondria of enterocytes through the activities of ornithine aminotransferase, pyrroline-5-carboxylate synthase, and ornithine carbamoyltransferase. Subsequently, L-citrulline enters circulation and is absorbed by the kidneys, where arginine synthesis occurs through the actions of argininosuccinate lyase and argininosuccinate synthetase.

### III. CATABOLISM OF ARGININE IN FISH

In mammals, various other metabolites are synthesized using arginine as a substrate by four sets of enzymes, which include arginine: glycine amidinotransferase (AGAT), nitric oxide (NO) synthases (NOS; 3 isozymes), arginases (ARG; 2 isozymes), and arginine decarboxylase (ADC). AGAT facilitates the catalysis of creatine production from arginine,

and the gene sequence of AGAT has been cloned in several fish species, such as zebrafish. Comparative analyses of AGAT gene sequences in 25 fish species have been conducted in relation to mammals, but information regarding its regulatory mechanism remains elusive. The synthesis of agmatine, catalyzed by arginine decarboxylase, has been identified in the mammalian brain and other organs, including the stomach and small intestine. However, our understanding of arginine decarboxylase in fish is currently lacking.

In fish, arginine undergoes catabolism by arginase, resulting in the production of products such as urea and ornithine. Hepatic arginase activity in fish is directly linked to the dietary arginine level. Various fish species, including rainbow trout, common carp (*Cyprinus carpio* L.), and Atlantic salmon (*Salmo salar*), have two identified isoforms of arginase known as Fish arginase-1 and fish arginase-2. These isoforms share similarities with mammalian cytosolic Type I arginase (hepatic OUC-related) and mitochondrial Type II arginase (nonhepatic), respectively. Studies on species like orange-spotted grouper and turbot have shown that higher dietary arginine levels lead to elevated hepatic arginase activities. In juvenile sea bass, surplus dietary arginine supplementation results in increased hepatic arginase activity and daily urea-N excretion. The mRNA expression and enzyme activity of arginase are positively correlated with dietary arginine supplementation, leading to higher urea excretion. However, it's crucial to note that approximately 85% of aquatic teleost fish excrete nitrogen wastes as ammonia, with only about 15% as urea. While Type I arginase is cytosolic and Type II arginase is mitochondrial in mammals, both arginases in fish contain a mitochondrial targeting sequence, with the subcellular localization of arginase activity in rainbow trout being predominantly mitochondrial. Studies suggest that arginases in ammonotelic animals are mainly localized in mitochondria, and cytosolic arginase I in fish may have evolved from an ancestral mitochondrial arginase II in vertebrates. The predominant mitochondrial localization of arginase activity in fish challenges the notion that its primary function is in the urea cycle. Ornithine, a product of arginase-catalyzed reactions, serves as a substrate for the synthesis of proline and polyamines (putrescine, spermidine, and spermine), influencing various growth and developmental processes due to their direct binding to DNA and RNA. Polyamines can impact fish immune responses and induce the production of reactive oxygen species (ROS). In common carp, arginase-2 gene expression and activity are upregulated by cAMP stimulation, likely playing a role in the alternative activation of fish macrophages. In the livers of fasted juvenile rainbow trout, enhanced arginase-2 expression contributes to increased ornithine synthesis, modulation of nitric oxide production, or related pathways. Moreover, arginase-1 and arginase-2 gene expression has been reported to upregulate in response to *Aeromonas salmonicida* infection. Although the specific roles of the two arginase isoforms in fish remain unclear, arginase in fish appears to be involved in the regulation of arginine and ornithine metabolism, polyamine production, immune responses, and other pathways unrelated to the urea cycle.

#### **IV. DEGRADATION OF ARGININE BY NITRIC OXIDE SYNTHASE**

Nitric oxide (NO), creatine, and various other metabolites are derived from arginine. Specifically, the biosynthesis of NO is facilitated by nitric oxide synthase (NOS), utilizing arginine as its sole substrate. Numerous studies have substantiated that NOS activity is significantly elevated with higher dietary arginine content compared to lower dietary arginine. Fish possess the capability to catalyze arginine through three sets of enzymes: (1) arginine:glycine amidinotransferase (AGAT), (2) nitric oxide synthases. In fish, three

isoforms of the enzyme nitric oxide synthase (NOS) are known to exist, namely, inducible (iNOS), neuronal (nNOS), and endothelial (eNOS) NOS, and (3) arginases (ARG), with two isozymes, arginase-1 and arginase-2, while the existence of arginine decarboxylase (ADC) in fish remains unknown. In the brain and neurons, the biosynthesis of nitric oxide is catalyzed by neuronal nitric oxide synthase (nNOS), playing a crucial role in embryological development. In goldfish (*Carassius auratus*) and brown trout (*Salmo trutta*), the distribution pattern of nNOS activity in different central nervous system regions has been determined, with the highest enzyme activity and protein expression of nNOS detected in the telencephalon and hypothalamus. NO production from nNOS and eNOS is relatively low and stable, whereas NO production from iNOS, induced by endotoxins, cytokines, or nutrients, can be significant. Consequently, iNOS is inducible, and its activity is largely dependent on extracellular arginine levels in both in vivo and in vitro settings. Increased arginase activity or a deficiency in arginine availability can lead to the uncoupling of NOS, causing it to produce superoxide instead of NO. This, in turn, stimulates arginase activity, inhibiting NOS and creating cellular oxidative stress, associated with endothelial dysfunction and cardiovascular diseases in mammals. NO serves as a potent vasodilator, enhancing blood flow to peripheral organs, facilitating increased oxygen and nutrient uptake. In the endothelium, NO diffuses into adjacent smooth muscle cells, activating guanylyl cyclase and increasing intracellular cGMP concentrations, thereby relaxing the muscle tissue. NO, produced by inducible NOS (iNOS), plays vital roles in immune function and pathogen clearance. However, excessive iNOS activity may result in detrimental overproduction of NO for the host cell. Similarly, NO in fish has been linked to cardiac function, as evidenced by the detection of nNOS-positive neurons in masu salmon (*Oncorhynchus masou*), indicating its modulation of somato-, viscerosensory, and visceromotor systems in the medulla. NOS is co-localized with Na<sup>+</sup> and K<sup>+</sup>ATPase in the gills of Atlantic salmon, suggesting a role for NO in ion transport. As a cofactor, NOS requires NADPH, and increased concentrations of reduced NADPH during smoltification suggest a role for NO in attenuating increased Na<sup>+</sup>, K<sup>+</sup>ATPase activity following seawater transfer. Arginine stimulates mitochondrial biogenesis through NO, regulating energy metabolism. NO-induced expression of peroxisome proliferator activator receptor (PPAR)-gamma coactivator1-alpha (PGC-1-alpha) further stimulates PPAR-alpha, promoting mitochondrial biogenesis and metabolism. In fish, the effects of arginine on both innate and adaptive immune responses have been demonstrated, where arginine may act through NO to combat pathogens, polyamines, direct effects on gene expression, or regulation of nutrient availability for immune cells through endocrine control. Inclusion of arginine in channel catfish diets has been correlated with increased survival when exposed to the bacterium *Edwardsiella ictaluria*. Arginine supplementation in diets improves macrophage killing and phagocytosis abilities in channel catfish, as confirmed in both in vivo and in vitro experiments, resulting in positive effects on the immune system. Furthermore, arginine enhances the proliferation of native T-cells and B-lymphocytes, along with increased hematocrit, hemoglobin, erythrocyte count, and lysozyme activity. Dietary arginine supplementation increases the respiratory burst after mitogenic exposure, correlating with increased NO production in head kidney leucocytes in Senegalese sole (*Solea senegalensis*). Exposure to both arginine and lipopolysaccharide (LPS) induces NO production from iNOS in head kidney macrophages. Therefore, substrate availability of arginine is crucial for producing an adequate immune response when needed. This underscores the importance of a sufficient dietary supply of arginine during pathogen exposure, considering that plasma arginine levels in fish decrease during stress. Arginine also increases the abundance of phosphorylated p38MAPK in Atlantic salmon head kidney and

liver cells in vitro, suggesting anti-inflammatory effects of arginine. Notably, even small concentrations of NO can protect cells from apoptosis and pathogens by activating heat shock proteins and inducing macrophage activity.

## V. REQUIREMENT OF ARGININE AND DEFICIENCY SIGNS

In animal species dedicating their entire energy to lifespan maintenance, growth primarily takes place during the juvenile period. Consequently, the dietary necessity for indispensable amino acids, such as arginine, in their adult phase is commensurate with the metabolic requirement.

However, in fish and other species that undergo continuous growth, the dietary requirement for arginine is primarily dictated by the growth imperative, as the maintenance requirement represents only about 10% or less of the arginine requirement for weight gain. In ureoletic mammals, ammonia serves as the ultimate waste product of amino acid catabolism, which can be converted into less harmful urea through the urea cycle. A deficiency in dietary arginine in carnivorous mammals invariably results in hyperammonemia (excessive ammonia in the blood), as the supply of dietary arginine is crucial for maintaining the capacity of the urea cycle to detoxify ammonia. However, in fish, a deficiency in dietary arginine does not lead to hyperammonemia, as the arginine requirement for the urea cycle in fish is relatively low, and fish can excrete most ammonia through their gills. Insufficient arginine intake in fish primarily results in delayed growth and impairment of immune capacity. Determining the arginine requirement in fish commonly involves evaluating growth performance. In fish feeds, the efficient utilization of certain protein sources, such as vegetable ingredients, depends on the proper supplementation of dietary amino acids to meet the specific requirements of each species. The arginine requirements for several farmed fish species have been established, ranging between 3.0% and 8.1% of dietary protein (Table 1). However, there is considerable variation in the reported dietary arginine requirements among different species, possibly due to differences in fish size and age, feeding levels, diet quality, laboratory conditions, and, notably, species differences. Additionally, factors such as the digestibility of dietary protein, differences in the utilization of amino acids from intact protein and crystalline form, and variations in fish size, feeding rate, dietary lysine and glutamine levels, rearing conditions, and diseases contribute to the observed differences in arginine requirements among species (Table 1). The effects of arginine on growth in fish are not solely attributed to optimized levels for protein synthesis in structural tissues but also involve the modulation of the somatotropic axis (e.g., growth hormone and insulin), mTOR signaling, and improvements in the antioxidant system and overall health. Consequently, other dietary components like somatotropic agents and antioxidants may influence arginine requirements in fish. Furthermore, dietary lysine and glutamine may also impact arginine requirements. The proper supplementation of arginine in fish diets should be based on the specific requirements of each fish species, taking into account the arginine provided by dietary ingredients. Inadequate balance may lead to deficiency or surplus signs, resulting in pathological issues. Deficiency in dietary arginine is associated with poor growth, anorexia, and high mortality. Elevations in plasma arginase activity, arising primarily from the hemolysis of red blood cells or liver damage, are linked to arginine deficiency, a phenomenon observed in various diseases in mammals but not yet studied in fish. Arginine deficiency can induce a range of physiological disorders, posing a threat to fish health.

**Table 1: Different Arginine Requirements in Numerous Farmed Fish Species**

Species	Crude protein (% of dry diet)	Arginine requirement (% of dietary protein)	Estimation model used	Criteria for requirement determination	Fish initial weight (g)	Dietary protein source	References
<i>Acipenser schrenckii</i> ♀ x <i>A. baerii</i> ♂	40	6.2	Broken-line	SGR; FER	3.6	FM; CGM; SB	Wang et al. (2017)
<i>Clarias gariepinus</i> x <i>C. macrocephalus</i>	40	4.5-5.0	Quadratic	LWG; SGR; FCR; PER	0.6	CS; GL; AA	Singh and Khan (2007)
<i>Carassis auratus</i>	31	5.3	Broken-line	SGR; FE; PRE	51	CS; AA	Tu et al. (2015)
<i>gibelio</i>	31	4.2	Broken-line	SGR; FE; PRE	147	CS; AA	
<i>Catla catla</i>	33	5.1	Quadratic	AWG; FCR; PER; PD	0.6	CS; GL; AA	Zehra and Khan (2013)
<i>Chanos chanos</i>	40	5.3	Broken-line	WG; FE; SR	0.7	CS; GL; AA	Borlongan (1991)
<i>Cirrhinus mrigala</i>	40	4.6	Quadratic	LWG; FCR; PER	0.6	CS; GL; AA	Ahmed and Khan (2004)
<i>Clarias gariepinus</i>	40	4.5	Broken-line	WG; SGR; SR	16.6	CS; GL; AA	Fagbenro et al. (1999)
<i>Cyprinus carpio</i>	48	4.3	Broken-line	WG SGR; PER; FE; PR	0.6	FM; RG; AA	Nose (1979)
	34	5.5	Quadratic	WG SGR; PER; FE; PR	6.3	FM; RG; AA	Chen et al. (2012a)
<i>Dicentrarchus labrax</i>	46	3.9	Broken-line	WG	2.1	MGM; HM; AA	Tibaldi et al. (1994)
<i>Epinephelus awoara</i>	43	6.5	Quadratic	WG; SGR; PPV; PER	4.2	BFM; SBM; WF	Zhou et al. (2012b)

<i>Epinephelus coioides</i>	50	6.1	Quadratic	WG	7.5	FM, SB, CGM, AA	Han et al. (2018)
<i>Heteropneustes fossilis</i>	40	4.0	Quadratic	LWG; FCR; PER; BPD	4.8	CS; GL; AA	Ahmed (2013)
	40	5.1-5.6	Broken-line	AWG; FCR; PRE; ERE	5.1	CS; GL; AA	Khan and Abidi (2011)
	38	3.9-4.3	Second-degree polynomial	LWG; FCR; PPV; SR	5.9	CS; GL; AA	Khan (2012)
<i>Ictalurus punctatus</i>	24	3.3-3.8 (glutamate added)	Broken-line	WG; FE; PER	11.4	CS; GL; AA;	Buentello and Gatlin (2000)
	24	4.2-5.0 (glycine added)					
	24	4.3	Broken-line	WG; FE	195-205	CS; GL; AA	Robinson et al. (1981)
<i>Labeo rohita</i>	40	5.8	Broken-line	SGR; FCR; SR	1.7	CS; GL; AA	Murthy and Varghese (1995)
	40	3.0-3.4	Broken-line and Second-degree polynomial	AWG; SGR; FCR; PER; PRE; ERE	0.6	CS; GL; AA	Abidi and Khan (2009)
<i>Lates calcarifer</i>	47	3.8	Broken-line	WG; FER; SR	2.6	FM; ZN; SM; AA	Murillo-Gurrea et al. (2001)
<i>Megalobrama amblycephala</i>	34	7.2	Second-order polynomial	SGR; FER; PER; PPV	2.7	CS; GL; FM	Ren et al. (2013)
	34	5.9	Broken-line	WG; SGR; FCR; PER	52.5	CS; GL; FM	Zhao et al. (2017)
	34	5.3	Broken-line	WG; SGR; FCR; PER	102		



<i>Micropterus salmoides</i>	45.9	4.1	Broken-line	WG; SGR; FCR; PER	25	ZN; AA	Zhou et al. (2012a)
<i>Morone saxatilis x M. chrysops</i>	33	4.4	Broken-line	WG; FE; SR	3.1	CS; GL; AA	Griffin et al. (1994)
<i>Oncorhynchus kisutch</i>	45	4.9- 5.5	Broken-line	WG; SGR; FCR; PER	0.9	FM; CS; GL; WG	Luzzana et al. (1998)
	50	4.0	Broken-line	WG; FE; NR	12.4	CS, GL, AA	Kim et al. (1992)
<i>Oreochromis niloticus</i>	28	4.8	Quadratic	WG; FCR; PRE; PER	3.0	CF; CG; RC; SB; FM; AA	Neu et al. (2016)
	28	4.2	Broken-line	WG	0.02	CS; GL; AA	Santiago and Lovell (1988)
	28	6.2	Broken-line	WG; FCR; SGR; PER; PRE	6.0	FM; SB; CGM; AA	Yue et al. (2015)
<i>Pagrus major</i>	50	4.5	Broken-line	WG; SGR; FCR; PER	13.3	WFM; SPC; AA	Rahimnejad and Lee (2014)
<i>Paralichthys olivaceus</i>	50	4.1-4.9	Broken-line	WG; FCR; SGR	1.9	CS; GL; AA	Alam et al. (2002)
<i>Pelteobagrus fulvidraco</i>	42	6.5	Quadratic	WG; SGR; FCR; PER	1.1	FM; CGM; SB; RSM	Chen et al. (2016)
<i>Perca flavescens</i>	33	4.9	Quadratic	WG; FE; SR	11	CS; GL; AA	Twibell and Brown (1997)
<i>Rachycentron canadum</i>	46	6.2	Second-order polynomial	WG; SGR; FER; SR	3.4	WFM; CGM; AA; WM	Ren et al. (2014)

<i>Salminus brasiliensis</i>	43	3.3	Broken-line and Second-order polynomial	WG; SGR; FCR	27	FM; AA	Dairiki et al. (2013)
<i>Salmo salar</i>	40	4.1	Broken-line	WG; NR	110	CS; CG; AA	Lall et al. (1994)
<i>Sciaenops ocellatus</i>	35	5.0	Broken-line	WG; PER; SR; FE	3.8	RDM; AA	Barziza et al. (2000)
<i>Sparus aurata</i>	42-44	5.55	Ideal protein	NR	4.6	FM; SFPC; AA	Peres and Oliva-Teles (2009)
<i>Sparus macrocephalus</i>	38	7.7-8.1	Broken-line and Second-order polynomial	WG; FER; SGR; PER; PPV	10.5	FM; SPC; AA	Zhou et al. (2010)
<i>Trachinotus ovatus</i>	43	6.3–6.4	Quadratic	WG; SGR; FCR; PER	18.8	FM; CGM; RM; PM; BYP; AA	Lin et al. (2015)

AE, ammonia excretion; BPD, body protein deposition; BYP, beer yeast powder; CF, corn flour; CGM, corn gluten meal; ERE, energy retention efficiency; FCR, feed conversion ratio; FM, fish meal; GLT, glutamate; GLY, glycine; NR, nitrogen retention; PA, plasma arginine level; PER, protein efficiency ratio; PM, peanut meal; PRE, protein retention efficiency; RC, rice; RGM, rice gluten; RSM, rape seed meal; SB, soybean meal; SFPC, soluble fish protein concentrate; SGR, specific growth rate; SPC, soy protein concentrate; WB, wheat bran; WFM, white fish meal; WG, weight gain; WGM, wheat gluten meal; WM, wheat meal; ZN, zein.

## **VI. INTERACTIONS OF ARGININE WITH LYSINE AND GLUTAMATE**

Nutritional and metabolic interactions involving arginine, lysine, and glutamate have been documented in various organisms. Arginine and lysine, being structurally similar amino acids composed of diamino monocarboxylic acids, share a common carrier for transportation across the brush border membrane. In the renal tubules of kidneys, lysine competes with arginine for reabsorption, diminishing the efficiency of arginine retention and potentially leading to negative implications for growth. Excess lysine adversely affects arginine utilization by impairing its efficiency. Conversely, glutamine, as a functional amino acid, holds a significant position and serves as the primary source of nitrogen and carbon in inter-organ amino acid metabolism, playing a crucial role in overall nutrient metabolism and fish health. Within the enterocytes, glutamine may be converted to citrulline via pyrroline-5-carboxylate. Subsequently, citrulline is transformed into arginine in the kidneys of healthy humans and transported to the liver, where it undergoes metabolism through the urea cycle or is utilized for the production of polyamines and creatine. In fish, observations of citrulline synthesis from glutamine and the sparing effect of glutamate on dietary arginine requirement have been reported. Supplementation of glutamine in an arginine-deficient diet significantly enhances feed efficiency and increases plasma citrulline and arginine levels in fish. The positive effects of glutamine on fish growth performance are particularly noticeable in diets deficient in arginine. However, it remains to be determined whether this holds true for all fish species. Additionally, further research is needed to understand the mechanisms, locations, and rates at which citrulline can be converted to arginine in fish. In fish, inter-conversion between arginine and glutamate is evident, with increased dietary arginine levels leading to elevated plasma levels of free citrulline, glutamate, and glutamine. Dietary administration of glutamine reduces the arginine requirement, and the formation of arginine from citrulline is inhibited by concentrations of glutamine below those encountered in tissue culture media or non-fish species' plasma. The presence of glutamine renders intracellular arginine rate-limiting for nitric oxide (NO) formation. Moreover, diets enriched with glutamine or arginine increase reactive oxygen species (ROS) production and modulate ROS output in neutrophils through a common pathway involving polyamine and NO synthesis. The synergistic effect of these amino acids can be explained by the essential role of glutamine as an energy substrate for lymphocytes and the proven positive impact of arginine on mitogenic stimulation and proliferation of T lymphocytes in various species, along with an increase in the number of cell surface receptors.

## **VII. NUTRIENT METABOLISM AND ENDOCRINE REGULATED BY ARGININE IN FISH**

Arginine induces activation of the endocrine system in fish through the stimulation of growth hormone (GH), insulin (INS), and insulin-like growth factor-I (IGF-I) release. Insulin and IGF-I, in turn, can initiate the TOR signaling pathway, facilitating muscle protein synthesis and regulating fish metabolism. Dietary supplementation of arginine in fish results in a significant increase in IGF-I mRNA expression in the liver and muscle, elevated secretion levels of GH and IGF-I in plasma, and a close correlation between GH, IGF-I levels, and fish growth performance. Furthermore, ascending dietary arginine levels lead to a substantial increase in serum insulin, GH, and IGF-I levels. Considering the pivotal roles of insulin and IGF-I as inducers of various signaling pathways, including AMPK and TOR, it is plausible that arginine promotes the activation of these pathways in fish. The heightened activation of the TOR signaling pathway has been demonstrated to enhance fish growth, as

supported by numerous studies indicating that dietary arginine supplementation activates the fish TOR signaling pathway and upregulates AMPK expression in the head-kidney, influencing the activation status of both AMPK and the TOR signaling pathway.

## VIII. CONCLUSION

In recent years, extensive research has been conducted on the dietary requirements of arginine in various fish species, leading to the identification of optimal dietary inclusion levels. Generally, carnivorous fish species demonstrate elevated arginine needs compared to omnivorous counterparts, and within the same fish species, the dietary requirement for arginine significantly diminishes with increased fish size. Despite possessing all genes encoding arginine biosynthetic enzymes, adult fish exhibit limited endogenous biosynthesis capacity, resulting in a heightened demand for dietary arginine. However, the enzymatic activities of these biosynthetic enzymes in fish are notably lower than those observed in mammals. Some inconsistencies exist regarding the ability of glutamine to serve as a source for arginine synthesis in certain fish species. The urea cycle in fish seems to lack a precise role in arginine synthesis, although arginine, especially at high concentrations, stimulates ureagenesis in select fish species. Arginine may contribute to ammonia detoxification by promoting the conversion of ammonia to urea. Variations in dietary arginine requirements across different species arise from factors such as size, feed ration, concentrations of other nutrients (e.g., glutamine and antioxidants), rearing conditions (e.g., stocking density, water flow rate), and external factors (e.g., stress, ammonia levels, and diseases). Despite these variations, arginine-induced growth modulation is attributed to positive health effects, including antioxidant and anti-stress effects, along with the somatotropic action of arginine. The extent to which increased insulin levels and the uptake of glucose and amino acids mediate the growth-promoting effects of dietary arginine remains unclear. Arginine stimulates the GH/IGF-1 axis, enhancing fish growth performance. The impact of dietary arginine on the NO system in fish is evident, and administration at specific levels benefits fish disease resistance in certain species. Mechanisms underlying these effects involve increased leukocyte numbers due to polyamines formation, elevated NO levels, pre-challenge upregulation of pro-inflammatory cytokines, and anti-inflammatory responses during pathogenic challenges. Although arginine activates various signaling pathways, including the AMPK and TOR pathways, in several fish species, the specific subcellular sensors responsible for activating these pathways remain unidentified, presenting a potential avenue for future research. Furthermore, appropriate arginine supplementation enhances fish immunity against environmental stress and pathogenic infections, while excessive supplementation may compromise fish disease resistance. Existing studies primarily concentrate on the regulation of fish antioxidant and innate immune responses, leaving a gap in understanding the effects of arginine on fish adaptive immunity—an intriguing area for future exploration. Particularly noteworthy is the novel approach to determining arginine requirements based on both fish innate and adaptive immune responses, deviating from traditional calculations based on optimum fish growth. Integrating this innovative approach into future studies promises a fresh perspective on arginine requirements in fish.

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