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Review Article

Arginine metabolism and its functions in growth, nutrient utilization, and immunonutrition of fish

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ABSTRACT

Fish have limited ability in endogenous biosynthesis of arginine. Arginine is an indispensable amino acid for fish, and the arginine requirement varies with fish species and fish size. Recent studies on fish have demonstrated that arginine influences nutrient metabolism, stimulates insulin release, is involved in nonspecific immune responses and antioxidant responses, and elevates disease resistance. Specifically, arginine can regulate energy homeostasis via modulating the adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) pathway, and also regulate protein synthesis via activating the target of rapamycin (TOR) signaling pathway. The present article reviews pertinent knowledge of arginine in fish, including dietary quantitative requirements, endogenous anabolism and catabolism, regulation of the endocrine and metabolic systems, and immune-regulatory functions under pathogenic challenge. Our findings showed that further data about the distribution of arginine after intake into specific cells, its sub-cellular sensor to initiate downstream signaling pathways, and its effects on fish mucosal immunity, especially the adaptive immune response against pathogenic infection in different species, are urgently needed.

1. Introduction

Arginine, also known as L-arginine (symbol Arg or R), is an α-amino acid that is mainly used for the biosynthesis of proteins. Arginine contains an α-amino group, an α-carboxylic acid group, and a side chain consisting of a 3-carbon aliphatic straight chain ending in a guanidino group, which results in arginine being a charged aliphatic amino acid at physiological pH (Armstrong et al., 2016). Arginine is an indispensable amino acid for fish because adult fish have low activities of arginine biosynthetic enzymes such as pyrroline-5-carboxylate (P5C) synthase, ornithine trans-carboxylase (OTC), and carbamoyl phosphate synthase (CPS) III (Andersen et al., 2016). The activities of other enzymes involved in the urea cycle such as argininosuccinate synthetase (ASS) and argininosuccinate lyase (ASL) have rarely been evaluated in fish, and only *N*-acetylglutamate synthase (NAGS) in zebrafish (*Danio rerio*) has been systematically studied and reported to be an intermediate form from microbial to mammalian NAGS on the evolutionary path (Caldovic et al., 2014). Adult fish mainly rely on their gills to excrete ammonia, thus they do not need to consume much adenosine triphosphate (ATP) for the urea cycle, but the enzymes involved in the urea cycle show relatively high activities during fish early development period (Wright, 2011). Arginine in fish serves as the substrate to synthesize many biologically active metabolites, including nitro oxide (NO), creatine, and polyamines (Han et al., 2018; Zheng et al., 2019). Ascending dietary arginine is also reported to significantly increase serum insulin (INS) and insulin-like growth factor-I (IGF-I) levels (Pohlenz et al., 2013; Han

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et al., 2018). Arginine activates adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK) to help the body save and use the available energy rather than synthesize lipid, and it also activates the target of rapamycin (TOR) signaling pathway to promote protein synthesis and myogenesis (Wang et al., 2015). Moreover, arginine has also been reported to exert multiple immune-modulatory functions, including the regulation of both the innate and adaptive immune response of fish, the inhibition of leukocytes apoptosis, and the regulation of fish resistance against bacterial disease (Hoseini et al., 2020). However, the effects of arginine supplementation on fish growth and immune responses may vary with arginine supplementation dosage, fish health condition, and environmental conditions (Azeredo et al., 2015). Deficiency in dietary arginine has been reported to result in reduced fish growth rate (Ball et al., 2007), and appropriate arginine supplementation can promote fish growth and immune responses, whereas surplus dietary arginine intake can inhibit fish growth and immune responses (Azeredo et al., 2015; Hoseini et al., 2019). Nowadays, with the increasing use of plant protein in fish diet, lysine becomes the first limiting amino acid (Gatlin et al., 2007). Dietary supplementation of synthetic lysine may affect the utilization and metabolism of arginine because of a possible antagonism between lysine and arginine (Zhou et al., 2011). Considering the important role of arginine in fish growth and immune responses, the present study reviews fish dietary arginine requirement, metabolism, and its regulation of the fish endocrine system, nutrient metabolism and immune response, and also proposes the direction for future research.

2. Arginine requirement in fish

In animal species that spend the majority of their lifespan on maintenance, growth mainly occurs within the juvenile period, thus the dietary requirement in their adult period for indispensable amino acids, including arginine, is equivalent to the metabolic requirement (Ball et al., 2007). However, in fish and other species that experience continual growth, dietary arginine requirement is mainly determined by the growth requirement because the maintenance requirement only accounts for approximately 10% or less of the arginine requirement by weight gain (Fournier et al., 2003). As is well known, ammonia is the final waste product of all amino acids catabolism, and it can be transformed into less toxic urea via the urea cycle in ureoletic mammals. Especially in carnivorous mammals, dietary arginine supply is regarded as critical for maintaining the urea cycle capacity for detoxifying ammonia (Visek, 1992) and dietary arginine deficiency always causes hyperammonemia (too much ammonia in the blood) (Kapila et al., 2001). However, dietary arginine deficiency would not cause hyperammonemia in fish because the arginine requirement for the urea cycle in fish is rather low and fish can excrete most ammonia via their gills (Ip and Chew, 2010). Insufficient arginine intake in fish mainly results in delayed growth and immune capacity impairment. A common approach to determine the arginine requirement in fish has been evaluation based on growth performance.

A previous review in 2007 reported that the arginine requirement of fish is relatively similar among fish species (1.20% to 1.50% of diet dry matter [DM] and 3.7% to 3.9% of crude protein [CP]) except Pacific salmon (2.04% and 5.4%, respectively) (Ball et al., 2007). However, later studies have indicated that much higher arginine levels are required (almost over 2.0% of DM and 6.0% of CP) in other fish species, especially in the carnivorous species (Zhou et al., 2012; Rahimnejad and Lee, 2014; Chen et al., 2016). These are systematically summarized in another review (Hoseini et al., 2020). For example, the optimal dietary arginine requirement in juvenile yellow grouper (*Epinephelus awoara*, approximately 4.23 g) was reported to be 2.8% of DM and 6.5% of CP, based on quadratic-regression analysis of fish growth (Zhou et al., 2012). A broken-line regression analysis of weight gain of juvenile red sea bream (*Pagrus major*, 13.3 ± 0.2 g) indicated that 2.37% arginine of DM and 4.74% arginine of CP were optimum (Rahimnejad and Lee, 2014). The quadratic regression analysis on weight gain of juvenile yellow catfish (*Pelteobagrus fulvidraco*) showed that 2.74% arginine of DM and 6.45% arginine of CP were optimal (Chen et al., 2016). Thus, the arginine requirement in fish is not stable and varies with different fish species. Generally, carnivorous species always have higher dietary arginine requirements than omnivorous fish. Table 1 presents the arginine requirements in representative fish species reported after 2010 in an order of increasing dietary protein levels. Table 1 shows that for most omnivorous species, dietary arginine requirement is lower than 2% of DM and 6% of CP when dietary protein levels are no higher than 40% ranking from Nile tilapia (*Oreochromis niloticus*) to Indian catfish (*Heteropneustes fossilis*), but for most carnivorous species, it is higher than 2% of DM and 6% of CP when dietary protein levels are no lower than 40% ranking from hybrid sturgeon (*Acipenser schrenckii*♀ × *Acipenser baerii*♂) to orange-spotted grouper (*Epinephelus coioides*). However, one previous study of Black sea bream (*Sparus macrocephalus)* has reported that the arginine requirement is 2.79% to 3.09% of DM and 7.7% to 8.1% of CP, respectively, when dietary protein is 34% (Zhou et al., 2010), which might be due to the excessively high test arginine range (2.54% to 2.88% of DM) with the defined minimum level even higher than the reported arginine requirement level in most other omnivorous species. Considering the important role of arginine in synthesizing protein for structural tissues and in activating the TOR signaling pathway as a signal, it is reasonable that carnivorous fish species, which require higher protein deposition and TOR signaling pathway activation, have higher arginine requirement than omnivorous species. In addition to the fish species, fish size has also been reported to be significantly correlated with arginine requirement. Dietary arginine requirement is estimated to significantly decrease with increased fish size. For example, the study of gibel carp (*Carassis auratus* gibelio var. CAS III) has shown that dietary arginine requirement is 1.64% of DM (5.3% of CP) for smaller fish (51.6 ± 0.3 g), and 1.29% of DM (4.2% of CP) for bigger fish (147.8 ± 0.5 g), respectively (Tu et al., 2015). In blunt snout bream (*Megalobrama amblycephala*), broken-line regression model on the basis of specific growth rate (SGR) has also shown that the optimal dietary arginine requirement is 2.03% of DM (5.97% of CP) for smaller fish (52.50 ± 0.18 g), but 1.79% of DM (5.27% of CP) for bigger fish (101.85 ± 1.85 g) (Zhao et al., 2017). The differential reported arginine requirement amounts for different-sized fish may be mainly contributed to the different protein synthesis rates. Additionally, arginine degradation also affects its requirement, although such an effect may be rather slight (Ball et al., 2007). Moreover, whether fish can reach the maximum growth potential during the experiment will also significantly affect the arginine requirement. Generally, fish with smaller initial body weight always show higher relative growth ratios during an experimental period of 8 or 12 wk. Therefore, fish growth performance should be carefully checked in an arginine requirement experiment, as reliable supports for aquafeed formula will only be supplied from data where maximum fish growth potential has been reached. The protein sources used in the experiment also significantly affect the calculated fish arginine requirement. The earliest studies mainly used purified or semi-purified diet with casein and gelatin as the main protein sources, and some studies after 2010 that we reviewed still used these 2 protein sources, such as the studies by Khan and Abidi (2011), Khan (2012), Ahmed (2013), Ren et al. (2013), Tu et al. (2015), and Zhao et al. (2017). However, the utilization of nutrients including arginine from casein and gelatin by

Table 1
Dietary requirement of arginine in representative fish species based on growth performance reported after 2010.

Species	Diet CP[1], % DM	IBW, g	Arg levels, % DM	SGR	Protein sources[2]	Criteria[3]	Model	Estimated Arg requirement (% of DM/% of CP)	References
Nile tilapia, *Oreochromis niloticus*	28	3.0	0.95 to 1.55	2.55 to 3.65	CF, CG, RC, SB, FM, AA	WG	Quadratic	1.36/4.8	Neu et al. (2016)
	28	6.0	0.85 to 2.24	2.55 to 2.79	FM, SB, CG, AA	WG	Quadratic	1.82/6.2	Yue et al. (2015)
Gibel carp, *Carassis auratus* gibelio var. CAS III	31	51.6	0.86 to 2.66	1.86 to 2.13	CS, AA	SGR	Broken-line	1.64/5.29	Tu et al. (2015)
	31	147.8	0.86 to 2.66	1.33 to 1.45	CS, AA	SGR	Broken-line	1.29/4.16	
Indian major carp, *Catla catla*	33	0.6	1.00 to 2.25	2.09 to 3.00	CS, GL, AA	WG	Quadratic	1.66/5.1	Zehra and Khan (2013)
Jian carp, *Cyprinus carpio*	34	6.3	0.98 to 2.45	3.15 to 3.30	FM, RG, AA	SGR	Quadratic	1.80/5.5	Chen et al. (2012a,b)
Blunt snout bream *Megalobrama amblycephala*	34	2.7	0.83 to 3.36	1.78 to 2.38	CS, GL, FM	SGR, FER, PER	Second-order polynomial	2.26-2.46/6.65 to 7.23	Ren et al. (2013)
	34	52.5	0.83 to 3.36	1.05 to 1.43	CS, GL, FM	SGR	Broken-line	2.03/5.9	Zhao et al. (2017)
	34	102	0.83 to 3.36	1.14 to 1.53	CS, GL, FM	SGR		1.79/5.3	
Black sea bream, *Sparus macrocephalus*	38	10.5	1.85 to 3.46	2.54 to 2.88	FM, SPC, AA	SGR, PER	Broke-line & Second-order polynomial	2.79-3.09/7.7 to 8.1	Zhou et al. (2010)
Indian catfish, *Heteropneustes fossilis*	38	5.87	1.11 to 2.27	1.63 to 2.69	CS; GL; AA	WG, FCR, PPV	Second-degree polynomial	1.51-1.66/3.97 to 4.37	Khan (2012)
	40	4.8	0.85 to 2.10	1.12 to 2.37	CS, GL, AA	WG, FCR, PER	Quadratic	1.62/4.08	Ahmed (2013)
	40	5.1	1.50 to 2.50	1.35 to 2.72	CS, GL, AA	WG, FCR	Broken-line	2.04 to 2.26/5.1 to 5.65	Khan and Abidi (2011)
Hybrid sturgeon, *Acipenser schrenckii*♀ × *Acipenser baerii*♂	40	3.63	1.74 to 3.54	4.14 to 4.45	FM, SBM, CG	SGR	Broken−line relationship	2.47/6.175	Wang et al. (2017)
Yellow catfish, *Pelteobagrus fulvidraco*	42	1.13	2.44 to 3.23	5.27 to 5.32	FM, SBM, RM, CGM	WG	Quadratic regression analysis	2.74/6.52	Chen et al. (2016)
Golden pompano, *Trachinotus ovatus*	43	18.8	2.05 to 3.58	2.17 to 2.68	FM, CGM, RM, PM, BYP, AA	WG, SGR, FCR, PER	Quadratic	2.74/6.4	Lin et al. (2015)
Yellow grouper, *Epinephelus awoara*.	43	4.23	2.01 to 3.27	2.56 to 2.87	FM, SBM	WG	Quadratic regression	2.80/6.52	Zhou et al. (2012)
Cobia, *Rachycentron canadum*	46	3.38	1.76 to 3.75	3.49 to 3.91	FM, CG, AA	WG	Second-order polynomial	2.85/6.2	Ren et al. (2014)
Orange-spotted grouper, *Epinephelus coioides*	50	7.52	2.13 to 3.74	3.02 to 3.35	FM, SB, CGM, AA	WG	Quadratic	3.05/6.1	Han et al. (2018)

[1] CP = crude protein; DM = dry matter.
[2] AA = amino acid premix; BYP = beer yeast powder; CF = corn flour; CG = corn gluten meal; CS = casein; FM = fish meal; GL = gelatin; PM = peanut meal; RC = rice; RG = rice gluten; RM = rapeseed meal; SB = soy-bean meal; SPC = soy-protein concentrate.
[3] FER = feed efficiency ratio; FCR = feed conversion ratio; IBW = initial body weight; PER = protein efficiency ratio; PPV = protein productive value; SGR = specific growth ratio; WG = weight gain.

fish is different from that from fish meal or plant proteins in practical aquafeed formula, thus it is of great importance to re-evaluate the arginine requirement in commercial feed formulation. Finally, different criteria and statistical methods have been adopted in dose−response arginine requirement studies. The arginine requirement in Indian catfish was determined by using fish with similar initial weight (4.8 to 5.1 g) and diets at the same protein level of 40% and with similar protein sources including casein, gelatin, and amino acid (AA) premix in 2 studies (Khan and Abidi 2011; Ahmed 2013), while their results varied with the different adopted models (quadratic or broken line model). Moreover, different arginine requirements were reported by Khan and Abidi (2011) due to the different adopted criteria with arginine requirement of 2.04% of DM (5.1% of CP) when weight gain (WG) was selected as criteria and that of 2.26% of DM (5.65% of CP) when feed conversion ratio (FCR) was selected as criteria. Although several new approaches such as single amino acid deletion, reduction, and diet-dilution techniques are also used for the amino acid requirement tests, the conventional dose−response method with different response criteria is still the most reliable method for an arginine requirement test. Generally, growth, nitrogen utilization, direct or indirect measurement of amino acid oxidation and metabolic responses were adopted as the criteria for the conventional method. However, we suggest that immune responses be taken into consideration in future studies because arginine significantly affects fish immune responses, which will be systematically further discussed in the follow-up section.

It should be noted that *N*-carbamylglutamate (NCG), a metabolically stable analog of *N*-acetylglutamate (NAG), shares effects similar to arginine because NCG could increase endogenous arginine synthesis by activating intestinal P5C and CPS I in mammals (Wu et al., 2004). Nile tilapia fed with a normal level of arginine show enhanced growth performance and feed utilization after dietary NCG supplementation (Cheng et al., 2015). Mirror carp fed with insufficient dietary arginine also exhibit better growth performance and feed utilization after dietary NCG supplementation of 0.12% or 0.16% (Wang et al., 2019). In Japanese seabass (*Lateolabrax japonicus*), dietary NCG supplementation (0.36%) promotes endogenous arginine synthesis and alleviates liver metabolism disease and hepatocyte apoptosis via the regulation of the extracellular regulated protein kinase 1/2 (ERK1/2)-TOR-S6K1 signaling pathway (Huang et al., 2019). These results might suggest that NCG

can act as a potential alternative for arginine in a practical diet with plenty of plant protein and supplemented synthetic lysine.

3. Arginine metabolism in fish

3.1. Arginine biosynthesis in fish

3.1.1. Arginine biosynthesis at early life stage of fish

Arginine can be synthesized in the urea cycle, and activities of enzymes involved in the urea cycle are relatively high especially during early development stage of fish (Fig. 1). The urea cycle in fish starts with the fixation of ammonia and glutamate into glutamine, and such a fixation is catalyzed by the mitochondrial enzyme glutamine synthase (GS) (Wright, 2011). Moreover, CPS I in mammals, the enzyme catalyzing the synthesis of carbamoyl phosphate in fish is CPS III obtaining the nitrogen source from glutamine rather than from ammonia (Shi et al., 2018). There is evidence that many teleosts are ammoniotelic as adults, but they are ureotelic at early life stages when they can produce a great amount of urea (Terjesen et al., 2001; Wright, 2011; LeMoine and Walsh, 2013). The embryos of zebrafish (Braun et al., 2009) and Atlantic cod (*Gadus morhua* L.) (Chadwick and Wright, 1999) are ureotelic with high activities of urea cycle enzymes. Rainbow trout (*Oncorhynchus mykiss*) are also reported to exhibit high activities of CPS III and ornithine transcarboxylase (OTC) during early development stages, in spite of this, their embryos are still ammoniotelic with at least 50% of nitrogenous waste as ammonia rather than as urea (Wright et al., 1995). These findings suggest that arginine synthesis is active during the early development period of fish (Wright and Land, 1998), however, we suggest that more data about the net biosynthetic ratio of arginine in the early developmental stage of fish be defined in future studies.

3.1.2. Arginine biosynthesis in adult fish

Although adult teleosts mainly rely on their gills to excrete ammonia, the genes encoding most enzymes involved in the urea cycle can be detected and the activities of some enzymes are also detectable in adult fish tissues (Wright, 2011). The activities of CPS III and other urea cycle enzymes in the muscle of tilapia are much higher than in the liver (Lindley et al., 1999). Similarly, in the African catfish (*Clarias gariepinus*), these enzymes are mainly localized to the muscle tissues, but not the liver (Terjesen et al., 2001). In adult trout, the maximum activity of CPS III is detected in the muscle tissue, but even maximum activity of CPS III in trout muscle (Buentello and Gatlin, 2000) is much lower (around 1/50,000) than the activity of CPS I in rat livers, suggesting that the biosynthetic ability of endogenous carbamoyl phosphate and arginine is rather low in adult fish. A recent study in rainbow trout also detected an increased arginine level in both muscle and blood plasma when citrulline was supplemented in diets, suggesting that citrulline supplementation may promote arginine synthesis (Clark et al., 2020a). Additionally, urea cycle enzyme activities have been reported to be significantly upregulated in some fish species under specific conditions, such as crowding or high pH (Laberge et al., 2009). All these results suggest that arginine synthesis is of physiological significance despite its low activity (Ball et al., 2007). Because fish are unable to endogenously synthesize arginine sufficiently to achieve optimal growth, additional arginine supplementation to fish diet is required (Korte et al., 1997).

3.2. Arginine catabolism in fish

In mammals, arginine serves as the substrate to synthesize many other metabolites by four sets of enzymes, including arginine:glycine amidinotransferase (AGAT), nitric oxide (NO) synthases (NOS; 3 isozymes), arginases (ARG; 2 isozymes), and arginine decarboxylase (ADC) (Morris 2016; Zou et al., 2019). AGAT catalyzes the creatine production from arginine, and the AGAT gene sequence has also been cloned in several fish species such as zebrafish (Wang et al., 2007). The gene sequences of AGAT in 25 fish species are compared with that of mammals (Borchel et al., 2019), but no information about its regulatory mechanism is available. Arginine decarboxylase, which catalyzes the production of agmatine, has been identified in the mammalian brain and other organs, such as the stomach, small intestine and aorta (Regunathan and Reis, 2000), while our knowledge about arginine decarboxylase in fish

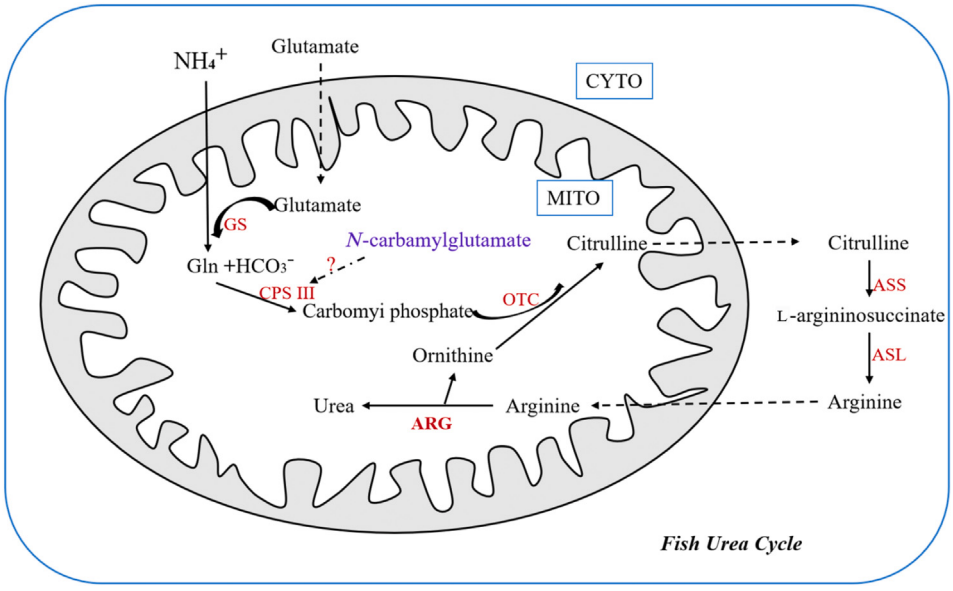

Fig. 1. Fish urea cycle consists of 5 key enzymes (carbamoyl phosphate synthetase [CPS III], ornithine transcarbamylase [OTC], argininosuccinate synthase [ASS], argininosuccinate lyase [ASL], and arginase [ARG]) and the accessory enzyme glutamine synthetase (GS) that combines glutamate and NH_4^+ to produce glutamine. In fish, 4 of these 6 enzymes are positioned within the mitochondria of hepatic and/or skeletal muscle cells and are active during early life stages but not as adults. Additionally, N-carbamylglutamate could increase arginine synthesis and share the similar role of arginine in fish although the mechanism is unknown. CYTO = cytoplasm; MITO = mitochondrion.

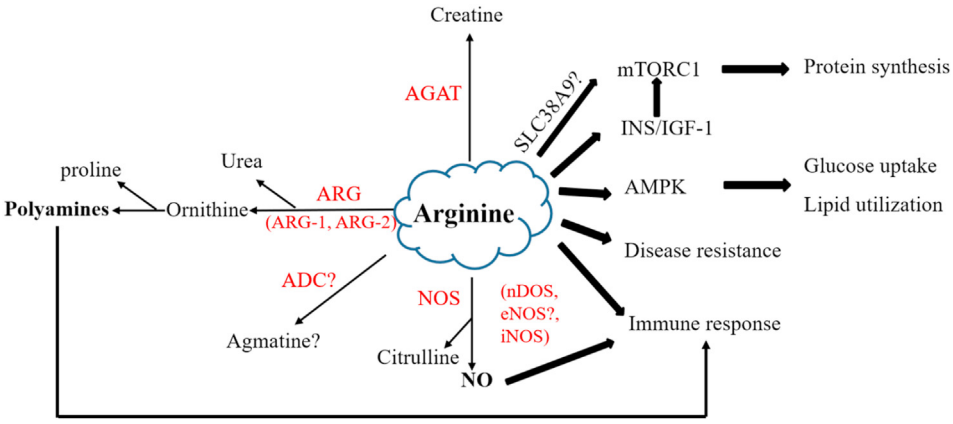

Fig. 2. Fish could catalyze arginine via 3 sets of enzymes: (1) arginine:glycine amidinotransferase (AGAT), (2) nitric oxide synthases (NOS; 3 isozymes, namely, neuronal NOS [nNOS], endothelial NOS [eNOS?, unsure about its existence in fish], and inducible NOS [iNOS]), and (3) arginases (ARG, 2 isozymes, namely, arginase-1 [ARG-1] and arginase-2 [ARG-2], whereas the existence of arginine decarboxylase (ADC) in fish is still unknown. Arginine can activate mTORC1 signaling pathway to promote fish protein synthesis, and regulate AMPK signaling pathway to increase fish glucose uptake and decrease lipid retention. In addition, arginine can also elevate fish disease resistance and immune responses, during which process nitric oxide (NO) and polyamines are involved in. mTORC1 = mechanistic Target of Rapamycin Complex 1; INS/IGF-1 = insulin/insulin-like growth factor-1; AMPK = adenosine 5′-monophosphate-activated protein kinase.

is absent (Fig. 2). Most previous studies focus on the arginine catabolized by arginase and NOS in fish, which will be discussed below.

3.2.1. Arginine catabolized by arginase

Arginine can be catabolized by arginase in fish into products including urea and ornithine and hepatic arginase activity in fish is directly correlated with dietary arginine level. Two isoforms of arginase have been identified in different species of fish such as rainbow trout (Wright et al., 2004), common carp (*Cyprinus carpio* L.) (Joerink et al., 2006), and Atlantic salmon (*Salmo salar*) (Andersen et al., 2015). Fish arginase-1 has a similar sequence to mammalian cytosolic Type I arginase (hepatic OUC-related), and fish arginase-2 shares a similar sequence to mammalian mitochondrial Type II arginase (nonhepatic). In orange-spotted grouper, hepatic arginase activities are found to be significantly higher in fish fed with higher levels of dietary arginine (3.48% of DM) than in fish fed with lower levels of dietary arginine (2.13% to 2.71% of DM) ($P < 0.05$) (Han et al., 2018). The highest gene expression of arginase I is detected in turbot (*Scophthalmus maximus* L.) fed with the highest level of dietary arginine (3.87% of DM) (Zhang et al., 2017), and a linear relationship between dietary arginine intake and urea excretion in rainbow trout and turbot is reported (Fournier et al., 2003). In juvenile sea bass (*Dicentrarchus labrax*), surplus dietary arginine supplementation (4.0%) to plant protein-based diets also increases both hepatic arginase activity and daily urea-N excretion (Tulli et al., 2007). Thus, dietary arginine supplementation significantly increases the mRNA expression and enzyme activity of arginase, resulting in higher urea excretion. However, it is important to note that entirely aquatic teleost fish excrete on average about 85% of their nitrogen wastes as ammonia and only about 15% as urea (Wood et al., 1989).

In mammals, Type I arginase is cytosolic while Type II arginase is mitochondrial. However, in fish, both arginases contain a mitochondrial targeting sequence (Joerink et al., 2006) and the subcellular localization of arginase activity in rainbow trout is largely mitochondrial (Wright et al., 2004). Several studies have provided evidence that arginases from ammonotelic animals are mainly localized in mitochondria and the cytosolic arginase I in vertebrates might evolve from a pre-existing ancestral mitochondrial arginase II (Srivastava and Ratha, 2013). The existence of arginase isoenzyme in mitochondria during rather early evolution also implies that the primordial function of arginase is regulation of cellular arginine and ornithine metabolism, unrelated to the urea cycle (Dzik, 2014). Considering that fish arginase activity is mainly mitochondrial, the main function of arginase in ammoniotelic fish might not be for the urea cycle (Dzik, 2014). Ornithine, another product of a arginase-catalyzed reaction, is a substrate for the synthesis of proline and polyamines (putrescine, spermidine, and spermine), which are involved in a variety of growth and developmental processes because they can bind directly to DNA and RNA (Romero-Calderon, 2006). Polyamines have been reported to affect fish immune response and they can induce the production of reactive oxygen species (ROS) (Reyes-Becerril et al., 2011). Moreover, arginase-2 gene expression and arginase activity in common carp were clearly upregulated by cAMP stimulation and likely involved in the alternative activation of fish macrophages (Joerink et al., 2006). The arginase-2 expression would be enhanced in the livers of fasted juvenile rainbow trout to enhance ornithine synthesis, modulate nitric oxide production or modulate related pathways (Wright et al., 2004). Additionally, the gene expression of arginase-1 and arginase-2 has been reported to be upregulated in response to *Aeromonas salmonicida* infection (Fast et al., 2009). Although the exact role of the 2 arginase isoforms in fish has been unresolved, arginase in fish might be involved in arginine and ornithine metabolic regulation, polyamines production, immune responses, and other pathways unrelated to the urea cycle.

3.2.2. Arginine degradation by nitric oxide synthase

Arginine also serves as the precursor of many metabolites such as creatine and nitric oxide. Especially, the NO biosynthesis is catalyzed by NOS with arginine as the only substrate. The NOS activity of juvenile red sea bream fed with 3.08% (of DM, the following percentages refers to that of DM unless indicated) dietary arginine was significantly higher than that fed with 1.42% dietary arginine (Rahimnejad and Lee, 2014). In juvenile yellow catfish, NOS enzyme activities showed a trend to firstly increase (1.59% to 2.74% dietary arginine) and then decrease (2.74% to 3.30% dietary arginine), and the highest activity was detected in fish fed with 2.74% arginine diet (Zhou et al., 2015). In gibel carp, both NO concentration in plasma and hepatic NOS activities were higher with increased dietary arginine level (from 0.54% to 1.76%) (Tu et al., 2015). In channel catfish (*Ictalurus punctatus*), extracellular

arginine availability significantly promoted the inducible NO production in macrophages in vitro (Buentello and Gatlin, 1999).

There are 3 isoforms of NOS in fish, namely, neuronal NOS (nNOS, or NOS1), inducible NOS (iNOS, or NOS2), and endothelial NOS (eNOS, or NOS3) (Cox et al., 2001). Neuronal nitric oxide synthase (nNOS) catalyzes the nitric oxide biosynthesis in the brain and neuron, which plays an important role in embryological development. The immunoreactivity of nNOS is detected in the olfactory receptor neurons of adult *Oreochromis mossambicus* (Singru et al., 2003). The distribution pattern of nNOS activity in different regional areas of the central nervous system has been determined in goldfish (*Carassius auratus*) and brown trout (*Salmo trutta*), and the highest enzyme activity and protein expression of nNOS are detected in telencephalon and hypothalamus (Virgili et al., 2001). Neuronal NOS-positive neurons are also detected in the medulla of masu salmon (*Oncorhynchus masou*), and NO is supposed to modulate its somatosense, viscerosense, and visceromotor systems (Puschina, 2012). The NO production in the endothelium is mainly catalyzed by eNOS, and NO is reported to modulate the ion transport in the kidneys and gills of trout (Tipsmark &Madsen, 2003) and in the opercular epithelium of killifish (*Fundulus heteroclitus*) (Evans et al., 2004). eNOS-like immunoreactivity has been detected with heterologous antibodies in zebrafish (Fritsche et al., 2000), tilapia (Cioni et al., 2002), and Atlantic salmon (Ebbesson et al., 2005). However, searching the completed fish genome projects reveals only iNOS and nNOS sequences, and thus eNOS is hypothesized to not exist in fish (Hyndman et al., 2006). Further studies are needed to illustrate the existence and role (if existing) of eNOS in fish. Both nNOS and eNOS are 2 constitutive NOS isoforms, which can only catalyze the production of low and stable levels of NO. However, iNOS is inducible and its activity is largely dependent on extracellular arginine levels both in vivo and in vitro. Our previous study revealed that extracellular arginine addition significantly elevated both the *iNOS* gene expression and NO production in fish leukocytes at 72 h post incubation (Zheng et al., 2019). Moreover, with prolonged inhibition of iNOS in juvenile salmon, heart rate was firstly increased and then decreased, suggesting that NO production might be associated with cardiac function in fish (Eddy, 2005a). NO also functions as an important component in the immune response and more knowledge about arginine immunonutrition will be further discussed in the following part.

3.3. Suggestions for future studies

As mentioned above, arginine serves as the substrate for many metabolites because it can be converted into urea and ornithine via arginase, or into NO and citrulline via NOS. However, the arginine distribution in different fish tissues after oral intake and arginine conversion into different metabolites in different fish cells after transport remains unknown, which significantly restricts the further study of modulation functions of arginine. In future studies, the LC-MS-based metabolomics and $^{15}N_4$-arginine tracing approach can be combined to quantitatively analyze arginine metabolism.

4. Endocrine and nutrient metabolism regulated by arginine in fish

4.1. Effect of arginine on fish endocrine system and cell signaling pathways

Arginine has been reported to activate the fish endocrine system by stimulating the release of growth hormone (GH), insulin (INS), and insulin-like growth factor-I (IGF-I). Insulin and IGF-I can further activate the TOR signaling pathway (Seiliez et al., 2008) to promote muscle protein synthesis and regulate metabolism in fish (Fig. 2, Fuentes et al., 2013). In channel catfish, dietary arginine supplementation significantly increases the mRNA expression of *IGF-I* in the liver and muscle and the secretion levels of GH and IGF-I in plasma, and the GH and IGF-I levels are closely correlated with fish growth performance (Pohlenz et al., 2013). In largemouth bass (*Micropterus salmoides*), hepatic IGF-I and pituitary GH mRNA levels are significantly upregulated with increased dietary arginine levels, and this upregulation exhibits a strong positive correlation ($r = 0.892$) with fish specific growth rate (Chen et al., 2012b). In gibel carp, GH and IGF-I concentrations in plasma are affected by dietary arginine levels, and the mRNA expression of pituitary *GH* is significantly elevated with increasing dietary arginine level (Tu et al., 2015). In orange-spotted grouper, the serum insulin and IGF-I levels are also significantly increased with ascending dietary arginine level (Han et al., 2018). Given that both insulin and IGF-I are important inducers of several signaling pathways, such as AMPK and TOR, potentially arginine can promote the activation of these signaling pathways in fish.

The increased activation of the TOR signaling pathway in fish has been reported to significantly promote fish growth performance (Wang et al., 2016). In young grass carp (*Ctenopharyngodon idella*), the relative mRNA expression of *TOR* in the gill was significantly upregulated with increased dietary arginine levels after an 8-wk rearing trial (Wang et al., 2015). Similarly, the relative mRNA expression of *TOR* and ribosome protein S6 kinase1 (*S6K1*) in blunt snout bream liver was significantly elevated with increased dietary arginine levels (Liang et al., 2016). In gibel carp, the relative expression of *TOR*, *S6K1*, *GH*, and *IGF-I* genes in the liver and muscle was significantly increased with increased dietary arginine levels (Tu et al., 2015). In Jian carp infected with *Aeromonas hydrophila*, the relative mRNA expressions of *TOR* and eukaryotic initiation factor 4E binding protein 1 (*4E-BP1*) in the head-kidney were significantly increased with increased dietary arginine levels (Martins et al., 2019). Thus, optimum arginine supplementation can activate the fish TOR signaling pathway. However, in grass carp, the relative gene expression levels of *TOR* and *S6K1* in the muscle show a trend to increase firstly (0.69% to 1.76% arginine), and then decrease (1.76% to 2.45% arginine) with increased dietary arginine levels (Wang et al., 2015). The expressions of genes involved in the TOR signaling pathway also display a similar trend in the intestinal epithelial cell of grass carp with increased arginine levels from 0 to 2.0 mmol/L in vitro (Chen et al., 2019). Moreover, the expression of *AMPK* in the head-kidney of juvenile blunt snout bream is also significantly increased with increased dietary arginine level (Liang et al., 2018). It should be noted that in most previous studies on fish arginine metabolism, evaluating activation status of AMPK and TOR signaling pathway based on mRNA expression may not reflect the real effect which should be conducted at post-translational level.

4.2. Effect of arginine on fish nutrient metabolism

Arginine has been reported to regulate fish carbohydrate uptake and lipid metabolism. For example, arginine has the potential to increase glucose utilization because it might stimulate glucose uptake by activating the translocation of glucose transporter-4 (GLUT4) (Andersen et al., 2016). The hepatic cells isolated from Atlantic salmon fed with high-level arginine diet also showed high glucose uptake (Andersen et al., 2014). In Atlantic salmon smolts (approximately 110 g), dietary arginine deficiency (1.1%) resulted in increased lipid retention, compared to dietary arginine levels ranging from 1.6% to 3.2% (Lall et al., 1994). Compared to fish fed with the control diet (2.88% arginine), fish supplemented with dietary arginine ranging from 3.17% to 3.55% exhibited a significantly

increased expression of carnitine palmitoyl transterase-1(CPT-1) in the liver, resulting in less fat accumulation in juvenile Atlantic salmon (approximately 5 g). However, surplus dietary arginine (3.7%) even increased the fat accumulation (Andersen et al., 2013). Another study of Atlantic salmon showed no influence of arginine (2.55% and 3.61%) on the mRNA expression of lipid metabolic genes (Andersen et al., 2014), which might be attributed to the excessively high level of arginine (3.61%) in the diet. In juvenile red sea bream, the minimum lipid content was found in dietary arginine supplementation of 2.22% and 2.54%, and such arginine levels also lead to optimal growth performance (Rahimnejad and Lee 2014). Dietary arginine supplementation in Nile tilapia also reduced high fat diet-induced fat deposition in the liver by regulating the expression of genes for lipid metabolism (Li et al., 2020). Therefore, the influences of arginine on fish lipid metabolism may be related to the arginine dosage and fish species.

In addition, arginine has also been reported to modulate fish amino acid metabolism. Dietary arginine supplementation (from 2.0% to 3.7%) significantly increased the basic level and 3 h post-prandial level of plasma ornithine in rainbow trout (Clark et al., 2020a), which was consistent with another study reporting that dietary arginine addition (5.64%) increased the ornithine level both in the plasma and liver at 18 h postprandial compared to the control (1.47%) (Fauzi et al., 2019). Moreover, dietary arginine supplementation significantly decreased the plasma citrulline content in rainbow trout (Fauzi et al., 2019; Clark et al., 2020a). Dietary arginine requirement could also be compensated with glutamine supplementation (Buentello and Gatlin, 2000). Much attention has been paid to the influence of dietary arginine supplementation on lysine metabolism in fish. Lysine is known as the first limiting amino acid in practical feed formula when plenty of corn and wheat gluten proteins are used as a protein source (Hardy, 2010), thus a large amount of synthetic amino acids, mainly including lysine and methionine, are added to fish diet (Li et al., 2009). Tracer accumulation studies show that antagonism between lysine and arginine does exist because these 2 amino acids compete for the same carrier-mediated pathways for transport across the brush border membrane (Murillo-Gurrea et al., 2001). The inhibition of arginine uptake by lysine is dependent on the relative concentrations of these 2 amino acids, whereas the inhibition of lysine uptake by arginine occurs regardless of lysine concentration. Surplus arginine supplementation over requirement resulted in growth depression of sea bass, which might be partially attributed to insufficient intestinal lysine uptake resulting from lysine–arginine antagonism (Vilella et al., 1990). Arginine utilization, indicated by the isotope activity in muscle tissue after abdominal injection of U–C^{14} arginine, was reduced in the muscle of Atlantic salmon fed with a high-level lysine diet (Berge et al., 2002). In vitro, arginase in Atlantic salmon hepatocytes was decreased with increasing lysine levels (Berge et al., 1998). However, the influence of lysine–arginine antagonism on the immune-nutritive functions of arginine still remains unknown.

4.3. Suggestions for future studies

Appropriate arginine supplementation significantly promotes fish growth performance, and such an effect is related to the influence of arginine on the fish GH/IGF-I endocrine system, TOR signaling pathway, and nutrient metabolism, among which the TOR signaling pathway is crucial. The specific mechanism by which arginine regulates the TOR signaling pathway remains unknown in fish, but the lysosomal membrane transceptor SLC38A9 is reported to activate the Rag–Ragulator complex, in turn to activate TOR in mammals (Abraham, 2015). The precise mechanism by which arginine regulates fish TOR signaling pathway, fish growth, and nutrient metabolism needs to be further investigated in future studies.

5. Arginine immunonutrition in fish

In addition to the regulatory functions in fish endocrine system and nutrient metabolism, arginine is also involved in regulating fish immune responses and disease resistance (Li et al., 2009). Compared to normal fish, fish under stress conditions such as environmental stress or pathogenic infection always experience nutrient metabolism reprogramming for better energy supply to sustain adequate immune response. This section will firstly review the reprogrammed arginine metabolism in fish under stress or infection to illustrate the importance of arginine in immune-competence, and then discuss the arginine regulatory roles in fish immune response and disease resistance.

5.1. Reprogrammed arginine metabolism of fish under stress or infection conditions

Amino acids are involved in the synthesis of proteins for immune responses and the regulation of key immune-related signaling pathways, suggesting their critical role in defense mechanisms (Andersen et al., 2016). Mammals can maintain the balance between anabolism and catabolism of arginine during moderate inflammation, and such a balance will be destroyed under severe inflammation conditions with the overriding of arginine catabolism on arginine anabolism (Bansal and Ochoa, 2003). In fish, the mechanisms of the reprogrammed arginine metabolism under multiple stresses remain largely unknown. With the application of advanced omics techniques in fish studies, the integrative analysis of enriched KEGG pathways allows identification of the metabolites involved in arginine and proline metabolism of fish suffering from pathogenic infection. For example, metabolomic studies reveal those hepatic metabolites of tilapia involving arginine and proline metabolism including γ-aminobutyric acid (GABA), fumaric acid, and L-proline are significantly decreased after *Edwardsiella tarda* infection (Peng et al., 2015). Similarly, metabolites involving arginine and proline metabolism of tilapia are also decreased after *Streptococcus iniae* infection (Ma et al., 2015). In zebrafish, the urea content in the whole-body homogenate is significantly decreased after being challenged with both ceftazidime-resistant and ceftazidime-sensitive *Vibrio alginolyticus* (Jiang et al., 2019). The urea concentration in crucian carp (*Carassius carassius*) is significantly decreased after infection with *E. tarda* (Guo et al., 2014). The decreased urea content during bacterial infection may indicate that arginine catabolism by arginase is significantly reduced. However, inconsistent results are reported in crucian carp, which shows a significant increase in arginine content in the serum metabolome after CyHV-2 infection ($P = 0.008$) (Tang et al., 2019). The differential results may result from different pathogens used in the studies, for example, a virus was used in the study by Tang et al. (2019) but bacteria were used in the study by Guo et al. (2014). In addition to metabolomics studies, transcriptome studies also confirm fish arginine metabolism reprogramming under stress. The transcriptome analysis of small yellow croaker (*Larimichthys polyactis*) indicates that arginine and proline metabolism is one of the enriched KEGG pathways of differentially expressed genes under heat and cold stress (Chu et al., 2020). Thus, fish can reprogram their arginine metabolism under pathogenic infection or other multiple stresses, during which process several signaling pathways

including TOR signaling and ERK signaling may be involved (Guo et al., 2019).

Nitro oxide production in vitro is commonly used as an index to illustrate fish arginine metabolism (Eddy, 2005b). The recombinant cytokines including TNF-α, IFN-γ, and IL-1β not only enhance the phagocytosis, but also increase NO production in fish macrophages and monocytes (Grayfer et al., 2009). With a multiplicity of infection (MOI) of 1:1 for 3 h, a high virulent strain (NUF251) of *E. tarda* induces the quicker release of NO and higher production level of TNF-α in the peritoneal macrophages of Japanese flounder (*Paralichthys olivaceus*) than a low virulent strain (NUF194) (Ishibe et al., 2009). In the macrophages of catfish, NO-dependent apoptosis is significantly induced after *Mycobacterium fortuitum* infection with MOI of 1:10 for 24 h (Datta et al., 2016). However, NO production is reported to be suppressed in other studies. For example, viral hemorrhagic septicemia virus (VHSV) infection (MOI of 1.78×10^{-3}) for 72 h suppresses NO production in the macrophage of turbot (Tafalla et al., 2001). These inconsistent reports may be due to different test fish species, different infection durations and pathogen dosages (Grayfer et al., 2009). All these results suggest that fish arginine metabolism is significantly affected during pathogenic infection or other environmental stresses, indicating the potential regulatory role of arginine in fish immune response and disease resistance.

5.2. Effects of arginine on immune response and disease resistance of fish

A recent study reviewed fish immuno-nutritional responses to indispensable amino acids including arginine, during which process TOR, NF-κB and Nrf2 signaling pathways were involved (Habte-Tsion, 2020). Appropriate arginine supplementation can promote fish immune response and disease resistance, partially via increased NO production, whereas surplus arginine may even decrease fish disease resistance, thus the arginine supplementation amount should be carefully evaluated in feeding practices. After summarizing the data reported in the literature after 2010, Table 2 presents the suggested dietary arginine levels which will promote fish immunity and disease resistance in several representative fish species.

The survival rate of channel catfish infected with *Edwardsiella ictaluri* was significantly increased by dietary arginine supplementation at 2.0% (Buentello and Gatlin, 2001). Similarly, the increased dietary arginine supplementation (ranging from 4.4% to 6.9%) also enhanced the disease resistance of Senegalese sole against *Photobacterium damselae* subsp. *piscicida* infection, increased respiratory burst activity, and promoted NO production in head-kidney leucocytes (Costas et al., 2011). Dietary arginine supplementation from 2.10% to 3.82% also significantly enhanced the innate immune responses, and decreased the cumulative mortality rate of juvenile turbot infected with *E. tarda* (Zhang et al., 2017). A reduction in the mortality rate of Nile tilapia fingerlings after *Streptococcus agalactiae* challenge and a boost in immune responses were observed in all supplementations of arginine (from 1.55% to 2.39%) in the diet (Vianna et al., 2020). Dietary arginine supplementation (from 2.08% to 3.48%) also modulated nonspecific immune responses in Indian major carp (*Cirrhinus mrigala*) exposed to hypoxia and reduced its mortality after *A. hydrophila* infection (Varghese et al., 2020). Arginine also elevated the survival rate of fish under ammonia-nitrogen stress. For example, the survival rate of juvenile yellow catfish after 72 h challenge with ammonia-nitrogen in 2.81% arginine supplementation group was significantly higher than that in 2.44% arginine supplementation group (Chen et al., 2016). However, the survival rate of golden pompano (*Trachinotus ovatus*) after challenge with *Vibrio harveyi* showed a trend to firstly increase and then decrease with the increased dietary arginine concentration ranging from 2.05% to 3.58% (Lin et al., 2015). The survival of rainbow trout fed with 2 dietary arginine levels (2.00% and 3.70%) showed no significant difference after *A. salmonicida* infection, which might have been due to a too high arginine addition level of 3.70% in the second group (Clark et al., 2020b). Similar results have also been found in European sea bass, whose immune status and disease resistance capability were impaired with surplus arginine supplementation (5.63% and 6.48%) (Azeredo et al., 2015).

Dietary arginine supplementation not only affects fish disease resistance capability but also affects fish inflammatory immune responses. Arginine was reported to protect fish against LPS-induced inflammation both in vivo and in vitro. For instance, arginine supplementation to both primary enterocyte culture media and Jian carp (*C. carpio* var. Jian) diet significantly inhibited the LPS-induced inflammatory response (Jiang et al., 2015). In Atlantic salmon head-kidney cells, arginine also inhibited the LPS-induced expression of pro-inflammatory genes (Martins et al., 2019). Our previous study also reported the inhibitory effect of arginine on the LPS-induced pro-inflammatory response (Zheng et al., 2019). Moreover, arginine supplementation was also reported to inhibit the apoptosis of fish leukocytes by enhancing NO synthesis (Zheng et al., 2019). In vivo, dietary arginine supplemention (3.65% of DM) also remarkably depressed the expression of *IL-8* and *TNF-α* of in gilthead seabream (*Sparus aurata* L.) during a skin wound challenge (Chen et al., 2020). However, other studies have reported the induction role of arginine during the inflammatory process. Arginine significantly increased the expression of pro-inflammatory genes in Jian carp, resulting in an elevated immune response and reduced mortality after *A. hydrophila* infection (Chen et al., 2015). However, the effects of dietary arginine on the inflammatory response of European sea bass was slight, mostly showing no statistical significance, which should be attributed to a too high sample variability (Azeredo et al., 2020). The fish inflammatory immune responses after arginine supplementation may differ depending on the virulence of pathogen and/or the dosage of LPS, and the optimal arginine supplementation level should be able to stimulate fish immune response without excessive response-induced damage. Considering that the exact regulatory mechanism of fish immune response remains largely unknown, the influence of arginine supplementation on the pattern recognition receptors (PRR)-mediated inflammation response remains to be further evaluated.

In fish, arginine may function in immune responses in multiple ways including producing NO and polyamines, directly modulating gene expression, and regulating nutrient availability of immune cells. A recent study also uses the shotgun proteomics technique to reveal the candidate serum proteins involved in fish immune responses after dietary arginine supplementation (Ramos-Pinto et al., 2020). Both in vivo and in vitro experiments with channel catfish showed that arginine supplementation improved the abilities of macrophage killing and phagocytosis, increased the lysozyme activity and erythrocyte number, and enhanced the proliferation of native lymphocytes after mitogenic exposure (Buentello et al., 2007; Pohlenz et al., 2012). Dietary arginine supplementation also increased the circulating monocyte number, promoted NO production, and improved humoral parameters of turbot (Costas et al., 2013). Moreover, dietary arginine supplementation in red drum (*Sciaenops ocellatus*) significantly increased the production of extracellular superoxide anion and neutrophil oxidative radical, along with the higher lysozyme activity (Cheng et al., 2011). As mentioned above, iNOS catalyzes the NO production upon various stimulations, and the iNOS activity is largely dependent on extracellular arginine level (Peranzoni et al., 2008). Under pathogenic exposure or ammonia-nitrogen stress, the arginine level in fish

Table 2
The optimum dosage of arginine for better immune response of several representative fish species based on reported articles.

Species	Arginine Dosage (% of DM/% of CP)	Evaluated innate immune parameters					Adaptive immune parameters	Survival rate against pathogens	References
		NO synthesis	Lysozyme activity	Hematological parameters	Antioxidant capacity	Regulation of inflammation			
Red sea bream, *Pagrus major*	2.54 to 3.08/5.08 to 6.16	Serum T-NOS activity	Activity in serum	Hematocrit & hemoglobin	SOD activity in serum		Ig in serum		Rahimnejad and Lee (2014)
Golden pompano, *Trachinotus ovatus*	2.65 to 2.98/6.16 to 6.93	Serum T-NOS activity	Activityin serum and liver		SOD activity in serum and liver			Survival rate against *Vibrio harveyi*	Lin et al. (2015)
Yellow catfish, *Pelteobagrus fulvidraco*	2.26 to 2.74/5.02 to 6.09	Serum T-NOS activity	Activity in serum	Red blood cell, white blood cell, hematocrit, hemoglobin	Serum SOD, CAT, GPX activity and MDA content			Survival rate against *Aeromonas hydrophila*	Zhou et al. (2015)
Nile tilapia, *Oreochromis niloticus*	3.56 to 4.38/9.89 to 12.17		Activity in plasma and spleen	Plasma hemolytic activity of complement system					Pereira et al. (2017)
Senegalese sole, *Solea senegalensis*	6.9/12.78	NO production in head-kidney leucocytes	Activity in serum	Respiratory burst activity		*MIP1-α* expression in head-kidney		Survival rate against *Photobacterium damseale* subsp. *piscicida*	Costas et al. (2011)
Yellow grouper, *Epinephelus awoara*	2.51 to 2.83/5.83 to 6.58	T-NOS activity in plasma and liver		Red blood cell, hematocrit, hemoglobin	SOD activity in plasma and liver				Zhou et al. (2012)
Indian Major Carp, *Cirrhinus mrigala*	2.78/8.00		Activity in serum	Albumin, globulin, and activity of antiprotease and myeloperoxidase				Survival rate against *Aeromonas hydrophila*	Varghese et al. (2020)
Jian carp, *Cyprinus carpio* var. Jian	1.27 to 1.61/3.74 to 4.74	iNOS activity in head-kidney and spleen	Activity in serum	Erythrocyte and leukocyte number		IL-1β in head-kidney	IgM in serum	Survival rate against *Aeromonas hydrophila*	Chen et al. (2015)

CAT = catalase; GPX = glutathione peroxidase; Ig = immunoglobulin; IL-1β = interleukin-1β; iNOS = inducible nitric oxide synthetase; MDA = malondialdehyde; MIP1-α = macrophage inflammatory protein-1-α; NO = nitric oxide; SOD = superoxide dismutase; T-NOS = total nitric oxide synthetase.

plasma is significantly decreased, indicating the necessity of adequate dietary arginine supply to maintain the sufficient immune response (Aragão et al., 2008). Dietary arginine in channel catfish significantly increased arginine level in plasma and NO production in activated macrophages, which contributed to an increasing survival rate after *Edwardsiella ictaluria* challenge (Buentello and Gatlin, 2001). Dietary arginine inclusion for Senegalese sole also increased NO production in leucocytes of head-kidney and enhanced respiratory burst upon mitogenic stimulation (Costas et al., 2011). Additionally, it has been reported that dietary arginine supplementation resulted in decreased plasma cortisol levels and enhanced immune responses in turbot under a 60-day chronic handling stress (Costas et al., 2013). It is important to note that both arginine and lipopolysaccharide (LPS) treatment resulted in increased NO production in head-kidney macrophages via iNOS (Buentello and Gatlin, 1999; Tafalla and Novoa, 2000). Small-dosage NO release can activate heat shock proteins and other macrophage functions to protect cells against apoptosis. Our previous study revealed that extracellular arginine supplementation significantly increased both *iNOS* mRNA expression and NO production in cultured fish leukocytes at 72 h post incubation, which was correlated with decreased apoptosis in head-kidney leukocytes (Zheng et al., 2019). However, excessive NO release can induce cell apoptosis by activating the endoplasmic reticulum stress pathway or other pathways (Mori, 2007). When dietary arginine is applied to improve fish health, it should not be ignored that fish immune responses are highly dependent on both the fish itself and the environment, and factors such as fish species, developmental stage, rearing densities, water temperature, dissolved oxygen, and other parameters of water quality should be taken into consideration.

5.3. Suggestions for future studies

As discussed above, arginine metabolism is significantly affected and reprogrammed under environmental stress or pathogenic infection, and appropriate arginine supplementation will benefit the immunity and disease resistance of fish. However, most existing studies have focused on fish systematic innate immunity, while little information about the regulatory roles of arginine in fish adaptive immunity is available. Moreover, fish intestine is not only the main tissue responsible for absorbing and utilizing arginine, but also functions in the defense against pathogenic infection along with other mucosal-associated lymphoid tissues, and IgT plays an important role in these tissues (Zhang et al., 2010; Xu et al., 2020), thus the influence of arginine on fish mucosal immunity should also be further investigated in future studies.

6. Conclusion

Plenty of studies in recent years have determined dietary arginine requirements in different fish species. Generally, carnivorous fish species exhibit higher dietary arginine requirements than omnivorous fish species, and dietary arginine requirement significantly decreases with increased fish size in the same fish species.

However, in some previous studies, the lack of a proper arginine test range has led to disputable research results, thus it is of great importance to set a reasonable arginine test range during experimental design in future studies. The limited capacity in endogenous biosynthesis results in high dietary arginine requirement in adult fish, which possess all the genes encoding arginine biosynthetic enzymes, but these enzymes show much lower activities than those in mammals. However, the precise arginine biosynthetic ability and the distribution of arginine after dietary intake in fish are rarely evaluated, thus future study is suggested to combine LC-MS-based metabolomics and $^{15}N_4$-arginine tracing approach to quantitatively define fish arginine metabolism. Although arginine is also reported to activate several signaling pathways including AMPK pathway and TOR pathway to regulate energy homeostasis and protein synthesis in several fish species, the specific subcellular sensors of arginine to activate these signaling pathways in fish have not been identified yet, which could be the direction for future research. Moreover, appropriate arginine supplementation will benefit fish immunity to fight against environmental stress and pathogenic infection, whereas surplus arginine addition may even impair fish disease resistance. The existing studies mainly focus on the regulations of fish antioxidant and innate immune responses, but little is known about the effects of arginine on fish adaptive immunity, which would be an interesting topic for future research. Especially, the determination of arginine requirement based on both fish innate and adaptive immune responses is different from the previous calculation of arginine requirement based on optimum fish growth, and this novel arginine requirement determination approach should be integrated into future studies, which will provide a new perspective.

Author contributions

Qingchao Wang: Writing the original manuscript; **Zhen Xu**: Reviewing the immune-nutritive part; **Qinghui Ai**: Reviewing the manuscript.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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