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·综述·

Advance in researches on arginine requirement for fish: a review

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Abstract: Arginine is an indispensable amino acid required by all fish species investigated to date. In recent years more research has been conducted to assess and confirm arginine requirement determined by growth performance. However, there are still many cultured fish whose arginine requirement needs to be qualified. Moreover, evidence has shown that large variation exists in the values estimated of arginine requirement among fish species. The paper reviews arginine requirement of fish based on available information in the literature. Factors influencing the estimates are described in details, and indices of assessing arginine requirement, as well as the interaction between arginine and lysine is also discussed.

Key words: fish; arginine; requirement

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Arginine is an indispensable amino acid required by all fish species investigated to date. In some plant protein- or casein - based diets arginine is considered to be among the most limiting essential amino acids[1-3]. Besides, arginine is involved in many metabolic pathways, such as protein synthesis, urea and ornithinine production, metabolism of glutamic acid and proline, and synthesis of creatine and polyamines[4-7], and, can also serve as a secretagogue and enhance the excretion of glucagons and insulinotropin[7,8].

The objective of the paper is to review advance in researches on arginine requirement in fish. This will certainly contribute to scientific formulation of cost-effective and environmentally friendly fish diets, and estimates of arginine requirement of other fish species. The paper will also explore the reason for large variation in arginine requirement and lay the foundation for further investigation of arginine metabolism in fish.

1 Arginine requirement

Published quantitative arginine requirements are summarized in Table 1. For the sake of uniformity, these values are expressed as a percentage of dietary protein. According to the table, there is a large range in the requirement values from the lowest value of 3.3% in channel catfish to the highest value of 6.8% in silver perch. Even in the same fish species, different researchers obtain rather different results. The large variation within and among fish species is not expected theoretically because whole-body protein of various fishes differs little in amino acid composition. Consequently, amino acid requirements of different

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species including arginine would not be expected to differ greatly. Based on these facts, it is essential to re-evaluate the requirement values of arginine for fish based on more reliable experimental methods.

Tab.1 Arginine requirement of some cultured fishes (expressed as a percentage of dietary protein)

fish species	requirement(%)	reference	fish species	requirement(%)	reference
Acipenser transmontanus	4.8	[16]	*Oncorhynchus mykiss*	3.5	[24]
Anguilla japonica	4.5	[17]		5.4-5.9	[32]
Bidyanus bidyanus	6.8	[18]		3.6-4.0	[33]
Catla catla	4.80	[19]		3.5-4.2	[34]
Chanos chanos	5.25	[20,21]		4.2	[35]
Clarias gariepinus	4.5	[22]		4.03	[36]
Clarias hybrid	3.6	[23]		4.1	[37]
Cyprinus carpio	3.8	[24]		3.5	[11]
	4.3	[17]		6.67	[38]
Dicentrarchus labrax	3.9	[25]		4.0	[39]
	4.6	[26]	*Pagrus major*	4.2	[40]
Hybrid striped bass	4.4	[27]		3.5	[41]
Salmo salar	4.1	[28]	*Paralichthys olivaceus*	3.4	[41]
	5.0- 5.1	[1]		4.08-4.20	[42]
Ictalurus punctatus	4.3	[29]	*Perca flavescens*	4.3	[43]
	3.3- 3.8	[12]	*Psetta maxima*	4.8	[26]
	4.2- 5	[12]	*Sarotherodon mossambicus*	4.0	[44]
Oncorhynchus tschawytscha	5.9	[30]	*Sciaenops ocellatus*	3.7	[45]
Oncorhynchus kisutch	6.0	[30]	*Sparus aurata*	5.0	[3]
	4.9-5.5	[31]	*Oreochromis nilotica*	4.2	[46]

2 Factors affecting estimates of arginine requirement

2.1 Species

The ornithine-urea cycle enzymes are reported to be present throughout a wide range of teleostean fish[9], so some species can synthesize arginine partially during the course of the urea cycle but amounts of synthesized arginine vary between species. For example, rainbow trout can utilize some intermediates like ornithine and citrulline for arginine synthesis[10,11]. Buentello and Gatlin[12,13] also suggested that endogenous synthetase of arginine from glutamate might be possible in channel catfish. Nevertheless, few reports are available on urea cycle enzyme activities in other fish and the significance of this metabolic pathway to contribute to satisfy some of the arginine requirement of each species may remain to be further investigated. Akiyama *et al*.[14] suggested that the variations in the essential amino acid requirements of different species possibly reflected true differences between phylogenetically distinct families or species because various fish species have the relative proportion of structured protein and the metabolic or physiological needs for specific amino acids[15].

2.2 Fish size and age

Generally speaking, young, rapidly growing fish need more arginine for protein synthesis and deposition, so have a higher arginine requirement than older, more slowly growing fish. Besides, arginine requirement of fish is related to arginine metabolism and activities of enzymes involving metabolism will change with age, but currently there are no specific trial or data to corroborate the influence because all of these researches conducted to determine arginine requirement are carried out under different experimental conditions influencing the estimated requirement.

2.3 Protein sources

When determining arginine requirement, high growth rate is important and can lead to a more reliable estimate of the requirement because growth is a physiological criterion to establish nutrient requirement[31]. Low growth rate usually results in artificial elevation of the estimated requirement[47]. However, the quantitative arginine requirements are usually determined by feeding graded levels of crystalline amino acid mix in purified or semi-purified diets with plant ingredients, such as, zein and maize gluten meal, as partial protein sources due to their deficiency in arginine[6-11,25,28,32,33,35,48]. In most of the experiments, poor growth rate has been obtained because many fish do not readily utilize crystalline amino acids and plant protein. Moreover, fish always have a relatively low digestibility coefficient for plant protein than fish meal, casein and gelatin, which may artificially elevate the estimated requirement. As a result, Luzzana *et al*.[31] corrected the actual arginine contents of the experimental diets by the apparent digestibility coefficients in an attempt to obtain an accurate estimate of the true requirement. Leaching of crystalline amino acid is another problem which can't be neglected. Leaching will reduce the amount of arginine available for fish growth and lead to over-estimation of arginine requirement of fish.

2.4 Dietary amino acid profile

As mentioned above, arginine requirement can partially be synthesized by urea cycle intermediates like ornithine and citrulline, so the dietary supply of these two amino acids can reduce arginine requirement. Besides, possible interactions between arginine, lysine and threonine in fish will also affect the requirement. Kim *et al*.[36] found that arginine requirement of fingerling rainbow trout was higher when dietary lysine exceeded the requirement value by up to 85%. Buentello and Gatlin[12] suggested arginine requirements of 3.3% – 3.8% of dietary protein with glutamate included in the diet, but of 4.2% – 5% of dietary protein with glycine inclusion in the diet.

2.5 Dietary protein level and energy density

Reasonable dietary protein is important for the estimates of arginine requirement because fish prefer to use protein as energy. For example, Kim *et al*.[36] determined an arginine requirement of 4.03% protein (or 1.4% diet) using 35% dietary protein for rainbow trout, but when using 24% protein he determined an arginine requirement of 6.67 % (or 1.6% of diet)[38]. Re-examining the two trials, Kim[38] thought that over 10% dietary protein was used for energy rather than for growth in the previous study. In contrast, Chiu *et al*.[34] thought the requirement for arginine increased as protein increased. However, corresponding data are missing in most publications dealing with arginine requirement.

Dietary energy concentration is considered to be one of the deciding factors for arginine requirement. Cowey[47] suggested that requirements (expressed in terms of dietary concentration) would be expected to increase as energy density of the diet increased. Rodehutscord *et al*.[11] also suggested that when comparing the value of arginine requirement derived from different studies, dietary energy concentrations should not be neglected. As a result, some researchers begin to express EAA requirement based on dietary energy level[11,17,35,49].

2.6 Feeding level and dietary electrolytes

Feeding levels influence the estimated value of arginine requirement, which has been demonstrated in several studies. The arginine requirement was about 4.2% of dietary protein when food intake was restricted, but the requirement decreased to 3.5% when fish were fed to satiation[34]. Luzzana *et al*.[31] also suggested an arginine level of 4.9% of the dietary protein for the satiation-fed groups and of 5.5% for the restricted-fed groups. Since the feeding regime adopted to evaluate the requirement for arginine should allow maximum growth rate, Cowey[50] recommended that in amino acid requirement studies the fish should be fed to satiation several times a day, which would allow a more reliable estimate. Regarding dietary electrolytes, Chiu *et al*.[34] suggested the arginine requirement tended to be higher when fish were fed diets containing the alkaline as compared to the acidic balance of mineral. Nevertheless, it remains to be shown to what extent dietary electrolytes may influence the requirement of arginine.

2.7 Water temperature and salinity

There is no convincing evidence that changes in water temperature and salinity *per se* affect the arginine requirement of fish. In general, water temperature can influence fish growth and arginine metabolism because activities of metabolic enzymes are related to water temperature. Marine or euryhaline teleosts have lower arginine requirement when kept in sea water relative to freshwater ones, the former being provided with potentially higher levels of the urea cycle enzyme activities which might ensure some biosynthesis of arginine[9].

2.8 Evaluating indices

At present, indices of evaluating arginine requirement include growth (weight gain, special growth rate) and feed efficiency[1,6,12,27,28,30–35,42,51], protein deposition[11,37,52], nitrogen retention[16,24], arginine retention[21] and various biochemical indices[1,6,28,42,53,54]. Because of variations of sensitivity of these parameters to graded levels of arginine, the criteria chosen influence estimates. For example, Berge *et al*.[1] obtained a requirement of arginine of 5.0% – 5.1% of the protein in Atlantic salmon based on growth and protein retention but a 4.8% based on free arginine in plasma and muscle tissue and urea in plasma.

2.9 Statistical analysis

Two kinds of curves have been used to determine arginine requirement, namely, broken line model and asymptotic curves. The former is based on the assumption that a growing animal will respond linearly to additions of a limiting indispensable nutrient until the exact requirement is met, after which no further growth response will be observed[55]. The model is widely accepted to determine arginine requirement in various studies[21,27,28,31]. When using asymptotic curves it was often defined by the point on the abscissa representing 95% of the values of the upper asymptote on the ordinate[1,56]. Generally speaking, the asymptotic model is regarded as the most appropriate for evaluating results from dose-response experiments[11,52,56]. However, if good fits of the data were obtained by two models, the estimated requirement values of arginine were very similar[55].

3 Deficiency or surplus symptoms

Symptoms of arginine deficiency consist of reduced growth, low feed efficiency and protein retention, as demonstrated in most fish species[1,12,20,25,31,32,29,42]. In addition, in channel catfish[12,29], rainbow trout[32] and Japanese flounder[42], high mortality and fin erosion have been reported as signs of arginine deficiency. It seemed that the development of deficiency symptoms was dependent both upon the species concerned and upon arginine deficiency in the diet, as pointed out in a report by Mazid *et al*.[57].

There are some differences in symptoms of arginine surplus reported in various fishes. Excessive arginine intake had no adverse effect on growth performance in channel catfish[29], sea bass[25] and turbot[39], but impaired growth and feed efficiency in Nile tilapia[16], milkfish[20], Atlantic salmon[1] and rainbow trout[39] because of arginine accumulation and hence toxicity in the tissue[20]. With salmonids[28,33,35], surplus arginine has been reported to ameliorate growth. This may be because post-prandial arginine can stimulate the release of growth hormone from pituitary[7].

4 Indices assessing arginine requirement

4.1 Weight gain, special growth rate and feed efficiency

These are the most widely used parameters in assessing arginine requirement of various fish species and often show a similar tendency of variation. At low dietary arginine level these values are low. Then they increase with increasing arginine contents and reached a plateau at higher level. Based on broken line analysis of weight gain and feed efficiency data, Buentello and Gatlin[12] determined arginine requirement of 3.3% and 3.8% of dietary protein in the diet with supplementation of glutamate for channel catfish, respectively.

4.2 Nitrogen retention and protein deposition

Some authors thought that weight gain was not the result of protein accretion alone, but also involved fat deposition, so they concluded that nitrogen retention and protein deposition were the most accurate and sensitive parameters to evaluate dietary amino acid requirements in fish[11,52,56,58]. Moreover, these parameters also take into account discrepancies in feed consumption and protein intake among different treatments. Based on analysis of 95% of the potential maximum protein deposition, Rodehutscord *et al*.[11] determined arginine requirement of 3.5 % of dietary protein for rainbow trout.

4.3 Ideal amino acid pattern

The method is based on studies that the essential amino acid pattern of the whole body tissue of fish generally correlates well with the quantitative amino acid requirements, so the EAA requirements are likely to be predicted using the A / E ratio (each EAA content / total EAA content including cystine and tyrosine). The method has been successfully used to predict other essential amino acid requirements after determining the lysine requirement through dose – response trials in several fish species, such as red drum and red sea bream[45], striped bass[59] and Japanese flounder[41]. However, some researchers suggested that other essential amino acid patterns, such as the amino acid patterns of whole hen egg[3] and fish eggs[60], would better reflect the EAA requirements than that of fish body. Alam *et al*.[61] also suggested that the amino acid pattern of brown fish meal better reflected the dietary EAA requirement pattern of juvenile Japanese flounder than that of whole body protein of juvenile Japanese flounder.

4.4 Biochemical indices

(1) **Free arginine in blood and muscle** The method lies in the observation that the free amino acid content remains low until the requirements for amino acids are met, and then sharply increased when excessive amino acids are fed. The indices reflect the net results of digestion, absorption and subsequent utilization[62], and are successful in confirming dietary requirements assessed by growth trial in some fishes, such as channel catfish[63], Atlantic salmon[1,28] and European sea bass[58], but also meet failure in other studies[6,27,29,33,35,42]. Its validity appears linked to the nature of the amino acid tested, the interactions between the different amino acids and the time elapsed between the meal and blood sampling[64]. Robinson *et al*.[29] suggested that serum levels of free arginine depended not only on dietary arginine concentration, but also on interrelations with other dietary amino acids. Other studies indicated that plasma-free arginine was responsive to dietary arginine in the low level, and then declined at the highest arginine[1,12,27] because a homeostatic mechanism for the regulation of free plasma arginine was present in fish[12]. The liver would be a likely site for this regulatory action due to the presence of relatively high arginase activity exerting control on circulating levels of arginine[1].

(2) **Urea in blood and excreted urea** The use of this biochemical index is based on the observation that urea production from the arginase pathway is related to arginine intake. Plasma urea concentration has been used to confirm the requirement value of arginine determined by growth study for rainbow trout[2,35], European sea bass[25] and Japanese flounder[42]. However, other authors suggest that post-prandial serum urea concentration was not helpful in confirming the requirement estimated by growth data, although it could be as sensitive as growth in determining the arginine requirement of the fish[33,35]. The origin of urea in teleosts is both from breakdown of purines[65] and from arginine degradation. The high correlation between arginine intake and urea-N excretion confirms the direct implication of dietary arginine degradation in ureogenisis[6, 39, 66].

(3) **Ammonia in blood and excreted ammonia** Supplementation of free arginine will lead to dietary amino acid imbalance, which will in turn lead to a reduced protein retention and consequently a higher production of ammonia[1]. This kind of phenomenon has been demonstrated in some studies. For example, Kaushik and Fauconneau[2] and Kaushik *et al*.[6] showed a positive relationship between different levels of arginine intake by rainbow trout and excreted ammonia, indicating the possibility of excreted ammonia as the parameter for confirmatory arginine requirement. Alam *et al*.[42] also reported that the excreted ammonia nitrogen is a useful index to prove arginine requirement determined by growth parameters, but it gave a slightly higher requirement value.

(4) **Arginase** Arginase is the last enzyme of the urea cycle, catalyzing the transformation of arginine into ornithine and urea. The enzyme shows the greatest activity in liver and kidney in almost all teleosts[67]. Alam *et al*.[42] reported that arginase activities in the liver were closely related to arginine intake in Japanese flounder and gave a confirmation of the requirement value by growth data. However, in other studies some authors insist that arginine requirement could not be determined from the activities of arginase measured in the liver or kidney of fish fed graded levels of arginine[1,28,33]. Walton *et al*.[33] was not able to detect any significant changes in vitro activity of liver arginase when the level of dietary arginine increased. So arginase validity need be further demonstrated in confirming arginine requirement.

(5) **Arginine oxidation** This technique is based on the general hypothesis that when arginine is limited or deficient in the diet, arginine will mostly be utilized for protein synthesis and synthesis of other essential components, and little will be accumulated in the plasma or be oxidized to carbon dioxide, whereas when the quantity of dietary arginine exceeds the requirement, arginine in plasma will increase and more will be used for oxidation. The technology of amino acid oxidation has been used to confirm lysine and tryptophan requirement of rainbow trout decided by weight gain by intraperitoneally injecting [U - ^{14}C] lysine and L - [$^{14}COOH$] tryptophan into fish body[68,69]. In other studies measurement of expired $^{14}CO_2$ after a tracer dose of L - (U - ^{14} C) arginine proved to be useful for assessing the arginine requirements of some fishes[6,28,33]. For example, Walton *et al*.[33] found a requirement of 1.8% in fish weighing 7g, based on the large increase in the oxidation of arginine. In contrast, Kaushik *et al*.[6] injected labeled arginine in the caudal vein and measured $^{14}CO_2$ excreted into water and air after 390 min in a metabolic chamber. The rate of oxidation of arginine was measured by the rate of excretion of $^{14}CO_2$. He found a notable increase in the rate of oxidation of arginine in fish fed the diets containing 1.6% to 2.0% of arginine, but a lower rate of arginine oxidation in fish fed diets with high level of arginine supplementation (above 2% diet).

(6) **Others** Dietary arginine can increase the release of most pancreatic hormones and growth hormone (GH), as demonstrated in different animals including fish[7,8,30,31,33,35,70]. This implies that the levels of pancreatic hormone and growth hormone will be useful indices assessing arginine requirement. However, Lall *et al*.[28] and Fournier *et al*.[39] could not demonstrate GH release connected to post-prandial action of arginine on the pituitary in Atlantic salmon and rainbow trout, respectively. So the mechanism of the insulinotropin of high arginine levels deserves more observations.

Another metabolic function for arginine that has received a great deal of attention in recent years is its conversion to nitric oxide by nitric oxide synthetase[71]. This occurs in hepatocytes but is probably a quantitatively minor reaction in liver under normal conditions. Competition between the formation of urea from arginine and NO synthetase involved in NO formation from arginine has been demonstrated in mammals. So arginase would have a major role in down regulatory NO synthesis. However, there is little information available on the effect of dietary arginine levels on NO production in fish although some evidence was found by Fournier *et al*.[39] that an inverse relationship between plasma urea levels and those of nitric oxide was present in rainbow trout.

5 Interaction between arginine and lysine

The antagonism between lysine and arginine is reported to be widely existent in higher animals, which is embodied in many aspects including competing for absorptive or re-absorptive sites in the intestine and renal tubules and by increasing amino acid degradation through interference with their normal intermediate metabolism[72-74]. In fish, however, the interaction between arginine and lysine is far from clear. Experiments with disproportionate lysine - arginine levels in fish diets have given different results and conclusions. Some authors concluded no dietary arginine – lysine antagonism per se existed in fish species, such as channel catfish[29], rainbow trout[36,75], hybrid striped bass[27] due to no significant differences in growth and feed efficiency among the treatments. In contrast, Kaushik and Fanconneau[2] and Kaushik *et al*.[6] reported a lysine-arginine antagonism occurred at the level of ureagenesis and excess lysine inhibited arginine catabolism and urea excretion in rainbow trout as in the case of mammals. Berge *et al*.[76] reported that arginine and lysine share a common carrier for transport across the brush membrane. Arginine had higher affinity for the common carrier and was the more potent inhibitor regardless of concentration. Lysine, however, had both a stimulatory and inhibitory effect on the uptake of arginine, depending on the relative concentration of the two amino acids[77]. The relative amounts of dietary arginine and lysine also influence their content in plasma and high levels of dietary lysine increase the level of lysine and reduce the level of arginine in plasma[78]. From these studies, it may be argued that arginine has an interaction with lysine at the level of uptake and metabolism but the kind of role is not necessary to reduce the growth performance of fish. Further studies indicated that the lysine-arginine antagonism could be affected by other factors, such as dietary electrolytes like Na^+, K^+ and Cl^- [34] and pH[72].

6 Conclusions

Despite many progresses in recent years, knowledge on the arginine requirement of fish is still far from complete and arginine requirement of many cultured fish remains unknown. Moreover, further repetition and verification with improved diets and experiments, as well as with admittedly unanimous criteria, are desirable to confirm assessments and establish more reliable arginine requirement of various fish species. Nutrient interactions between arginine and other amino acids as have been demonstrated in several literatures certainly need to be explored further. Studies to explore the arginine metabolism should be given more attention.

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鱼类精氨酸需求研究进展

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摘要：精氨酸是迄今研究的所有鱼类的必需氨基酸。近年来，在确定鱼类精氨酸需要量方面取得了巨大的进展。然而，仍然有许多养殖鱼类对精氨酸的需求有待研究。在已有的报道中，鱼类精氨酸需求存在种内和种间差异，而且这种差异较大。这篇论文综述了鱼类精氨酸需求的研究进展，包括五方面的内容，即鱼类对精氨酸的需要量，饲料中精氨酸含量对鱼类的影响，影响鱼类精氨酸需要量的因素，评价鱼类精氨酸需要量所采用的指标以及精氨酸和赖氨酸之间的相互关系。

关键词：鱼；精氨酸；需求

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