Arginine improves the immunocompetency, disease resistance as well as resistance ability against stress tolerance of fish

Dinh Hai Nguyen
CJ Bio, Vietnam

Introduction

In response to the increase in the demand for animal protein by a growing global population, producers are compelled to significantly increase production. Aquaculture is one of the fastest developing growth sectors in the world and Asia presently contributes about 90% to the global production (Sahu et al., 2008). The cultivation of aquatic animal has been increasing widely with large scale intensive culture systems continuing to appear, which results to that fish are exposed to stressful conditions, problems related to diseases outbreaks, which results in severe financial losses due to high mortality.

Antibiotics were used for a long time in aquaculture to prevent diseases. However, this caused various problems such as the presence of antibiotic residues in aquatic animal tissues, the generation of bacterial resistance mechanisms, as well as an imbalance in the gastrointestinal microbiota of aquatic species, which affected consumers' health (Nakano, 2007).

Besides many potential alternatives such as organic acids, organic minerals, bacteriophages, probiotics, and prebiotics, nutritional status is considered one of the most important factors that influence the ability of aquatic animal to resist diseases. Previous studies have suggested that arginine is not only being an essential amino acid in all fish species but also a functional amino acid, improve the outcome from disease (Robinson et al., 1999; Wu et al., 1999; Wu et al., 2018) as well as increase resistance against stress tolerance (Chen et al., 2016; Wu et al., 2018). In addition, to maintain the optimal growth, arginine participates in the biosynthesis of peptides such as the immunologically important thymosin, thymopeptin, tufsin as well as proteins such as histones (Brittenden et al., 1994). Moreover, arginine is the sole precursor for nitric oxide synthesis (Reyes et al., 1994; Akarid et al., 1995). This highly reactive molecule has been reported to play a central role in tumor cytotoxicity by activated macrophages and has been shown to mediate the macrophage's killing of various microorganisms. Microbial targets of nitric oxide include parasites, fungi, bacteria, and viruses (Hibbs et al., 1987; De Groote and Fang, 1995). It is therefore this article provides an overview of the efficiency dietary arginine administration on immunity and resistance of fish to pathogenic bacteria as well as stress agents.

L-arginine metabolism

As one of the essential amino acids for fish, arginine takes part in many metabolic reactions in animal bodies, such as the synthesis of protein, carbamide and ornithine, the metabolism of glutamic acid and proline, the synthesis of creatine and polyamine, and the excretion of insulin and glucagon (Zhi et al., 2004; Figure 1). Arginine is a multi-functional amino acid being involved in reduction of oxidative stress (Wu et al., 2018), and fat accumulation (Rahnama, 2010) as well as enhancing immunity system as a trend which receives rising interest in the pathogenic microorganism resistance (Buentello and Gatlin III, 2001; Vianna et al., 2020).

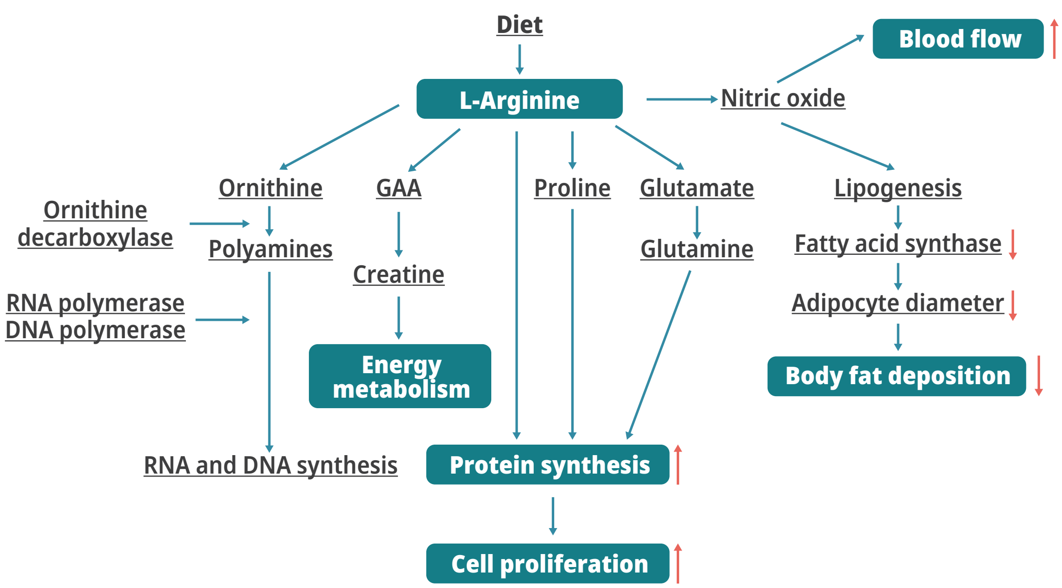


Figure 1. Role of L-arginine in different metabolic pathways (Adapted from Fouad et al., 2012; Khan, 2019).

Arginine improves stress tolerance resistance and disease resistance

Under intensive conditions, fish have to expose to many stressful conditions, for example under the high density of factory farming water, the ammonification of bait and fish excreta will produce large amounts of ammonia nitrogen, which will result in feed intake decreased, growth reduction and immune suppression. The excessive ammonia nitrogen in the environment can also damage the gill, liver, kidney and other organs of the fish (Zhang et al., 2015). Besides, fish are also exposed to many problems related to diseases outbreaks. For example, while streptococcus is regarded as the worst disease for tilapia worldwide, and Streptococcus agalactiae has become a major pathogen for tilapia (Mian et al., 2009; Ali et al., 2010; Li et al., 2014; Soto et al., 2015; Yang et al., 2018), Edwardsiella ictaluri is the causative agent of enteric septicemia of catfish (Hawke et al., 1981) and is one of the most devastating pathogens that infect channel catfish Ictalurus punctatus (MacMillan, 1985; Plumb, 1994). The high morbidity and mortality associated with pathogenic microorganisms is a major threat to fish farming and lead to economic losses.

Previous studies have shown that dietary arginine supplementation can improve immunity in many fish species, such as red drum (Cheng et al., 2011), hybrid striped bass (Cheng et al., 2012), yellow catfish (Zhou et al., 2015), golden pompano (Lin et al., 2015), Senegalese sole (Costas et al., 2011), Jian carp (Chen et al., 2015), Channel Catfish (Buentello and Gatlin III, 2001), and tilapia fingerlings (Vianna et al., 2020), ammonia-nitrogen stress (Chen et al., 2016) and oxidative stress of fish (Wu et al., 2018) .

It is reported that arginine may be more effective as an immunostimulator thanks to the nitric oxide which not only oxidizes bacterial cell membranes and regulate variety of immune cells but also inactivates enzymes of the mitochondrial respiratory chain, literally starving bacterial cells to death (Lancaster, 1992). The beneficial effects of arginine also may be noted as increased total leukocyte counts because arginine is an important substrate for biosynthesis of polyamines that are essential for cell proliferation (Narita et al., 1995). During bacterial exposure, the immune system requires the host to synthesize new proteins such as lymphokines, complement, and antibodies (Lall and Olivier, 1991). Supplementation with arginine may stimulate macrophages to secrete cytokines mediated by polyamines because arginine has been shown to be a polyamine precursor (Barbul, 1995; Moinard et al., 1999).

Arginine increases stress tolerance resistance of fish

Chen et al. (2016) evaluated the effects of dietary arginine levels on resistance ability against ammonia-nitrogen stress in juvenile yellow catfish. After a 56 d feeding trial with 5 graded levels of arginine (2.44%, 2.64%, 2.81%, 3.01% and 3.23% of diet) for growth performance calculation and other index determination, fourteen fish were randomly selected from each tank for an ammonia-nitrogen stress test using ammonia chloride. During the test, the fish were not fed. Ammonium chloride solution was poured into each tank of 150 L to set the level of total ammonia nitrogen at 100 mg/L, and the concentration of un-ionized ammonia was 2.65 mg/L (pH = 7.52; water temperature = 30.5^{0}C). During the stress test, water temperature, pH level, and ammonia nitrogen concentration were determined every 6 h, and adjust the concentration of nonionic ammonia according to the results using ammonium chloride solution. Mortality was recorded every 6 h during the period of 72 h stress test and dead fish were removed. The cumulative mortality rate (CMR) was calculated as follows: CMR (%) = 100 x number of death fish after stress test/number of fish before stress test. After the fish were challenged to ammonia-nitrogen for 72 h, their cumulative mortality rate in 2.81% group was significantly lower than that in 2.44% group ($P < 0.05$). The results suggested that dietary arginine level at 2.81% could optimize anti-ammonia-nitrogen stress ability of juvenile yellow catfish (Figure 2).

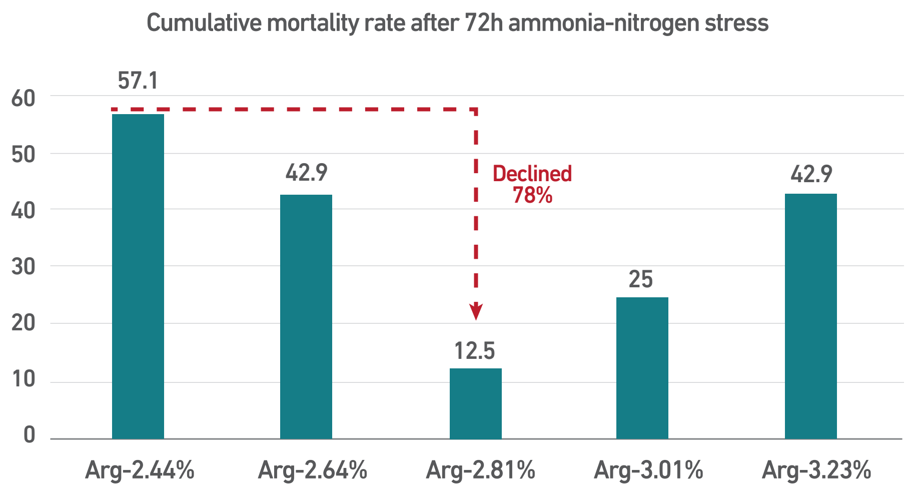


Figure 2. Effects of dietary arginine levels on the cumulative mortality rate after 72 h ammonia-nitrogen stress test of yellow catfish (Pelteobagrus fulvidraco) (Adapted from Chen et al., 2016)

An other reccent study also evaluated the effects of dietary arginine levels on resistance ability against oxidative stress by the addition of $CuSO_4$ to water in hybrid grouper (Wu et al., 2018). After 8 weeks feeding trial with 5 graded levels of arginine (1.9%, 2.2%, 2.7%, 3.1%, 3.8%, 4.1% and 4.7% of dry matter for growth performance and feed and other index determination, all remaining fish were fed their prescribed diets for 2 days and then exposed to 4.5 mg Cu (II)/l water for 36 h by the addition of $CuSO_4$ to water. The dose of Cu (II) exposure used in this study was found to induce oxidative stress in a preliminary experiment. During the challenge test, the water recirculation of the aquarium system was stopped by turning off the pump. The survival rate per aquarium was recorded, and then three fish per aquarium were randomly selected and individually sampled as above to obtain serum, liver and head kidney. The trial results shown that after exposure to 4.5 mg Cu^{2+} /l water for 36h, fish fed 1.9 and 2.2 % arginine levels had lower survival ratios than fish fed higher (2.7 – 4.7 % dietary) arginine levels (Figure 3). The results from this study further showed that the mode of action by which arginine acts on the hybrid grouper's innate immunity is possibly by increasing serum lysozyme activity and IgM concentrations, together with stimulating the Nrf2-Keap1 signaling pathway and HSP70 expression in head kidney; However, before challenge, fish survival ratios and serum lysozyme activity showed no remarkable variations among all experimental treatments after they were fed experimental diets for 8 weeks. These indicated that the activation of the immune system by suitable arginine supplementation in hybrid grouper mainly occurred when the fish was subjected to Cu stress.

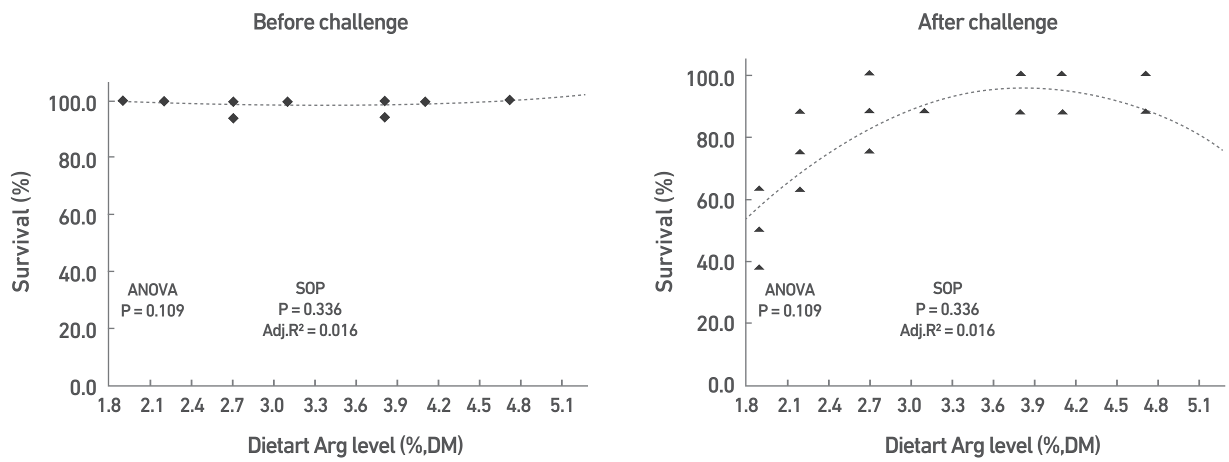
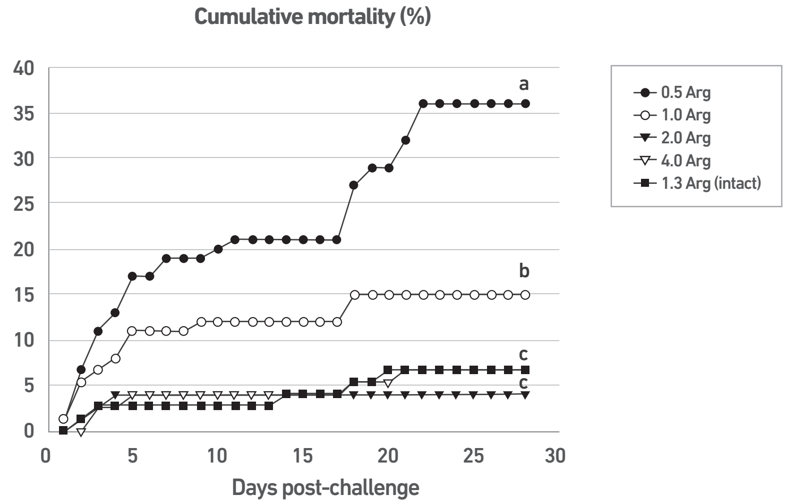


Figure 3. Survival rate of hybrid grouper juveniles fed different dietary arginine (Arg) levels for 8 weeks before/after exposure to 4.5 mg Cu(II)/l water for 36 h (n = 9) (Wu et al., 2018)

Arginine improves disease resistance of fish

Buentello and Gatlin (2001) evaluated the effects of dietary arginine on resistance of channel catfish to exposure to *Edwardsiella ictaluri*. After 15 days feeding trial with four experimental diets were prepared by adding graded amounts of L-arginine·HCl to provide arginine at 0.5, 1, 2 or 4% of the diet (1.8, 3.6, 7.1 and 14.3% of dietary protein, respectively). And a purified intact-protein diet containing 28% crude protein and 1.3% arginine (4.6% of dietary protein) from casein and gelatin, for the blood sample collection, fish were exposed to *E. ictaluri* by lowering the water level in all 18 aquaria to 2.4 L and adding 10 mL of bacterial broth to yield approximately 1.3 × 10^8 colony-forming units (CFU)/mL. After a 90-min static exposure, water flow was resumed, and the tanks were allowed to refill to the original volume. Morbidity, mortality, and cause of death were monitored and recorded daily. Dead or dying fish from each set of three aquaria per diet were necropsied daily. Bacterial isolates from the trunk kidney were identified from biochemical characteristics (Hawke et al., 1981) to confirm the cause of death from E. ictaluri. The experiment was terminated 28 d after bacterial exposure. Mortalities were recorded for all diets as soon as 2 d after exposure to bacteria, whereas the control fish did not exhibit any mortality throughout the experiment. The study results shown that fish fed the diet with arginine at 1.8% of protein began to die on day 1 post exposure and continued to die until termination of the experiment. In contrast, fish fed the diet with arginine at 7.1% of protein showed a significantly (P = 0.0001) higher survival than did fish fed the diet with arginine at 1.8% of protein, and no mortalities were recorded after day 4. There were no significant (P > 0.05) differences in the mortality of fish fed diets with arginine at 14.3% of protein or 4.6% from intact protein. At day 21, fish fed diets with arginine at 4.6, 7.1, and 14.3% of protein showed approximately 94% survival, whereas those fed diets with arginine at 1.8% and 3.6% of protein exhibited survival of less than 64% and 85%, respectively (Figure 4). The challenge test gusset the dietary arginine, at twice the level required for optimal growth, enhanced survival of channel catfish exposed to E. ictaluri. Because exoantigens from E. ictaluri attract activated macrophages to infected kidneys, spleen, and liver (Baldwin and Newton, 1993), it is likely that enhanced survival was due, in part, to an increased capacity of macrophages to destroy pathogenic bacteria.

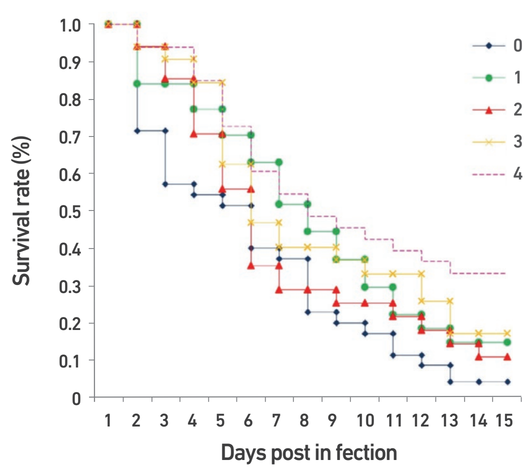
The effect of arginine was again reinforced that arginine may have provided the needed substrate to allow nitric oxide to exert its microbicidal activity as demonstrated in previous studies (Granger et al., 1988; Leone et al., 1991; Arias et al., 1997), thus, preventing the spread of *E. ictaluri* to other tissues. This enhancement of macrophage microbicidal ability by arginine is further supported by the studies of Adams et al. (1990) in which the killing of engulfed *Toxoplasma gondii* by INF-gamma-activated macrophages was dependent on L-arginine and inhibited by N^G-monomethyl-L-arginine.



Lines with different letters (a—c) are different (P < 0.05). Symbols represent means of three replicate tanks per treatment. Pooled SE is 1.8. The F-statistic is 0.0001 for the dietary arginine effect; Arg = arginine Buentello and Gatlin (2001).

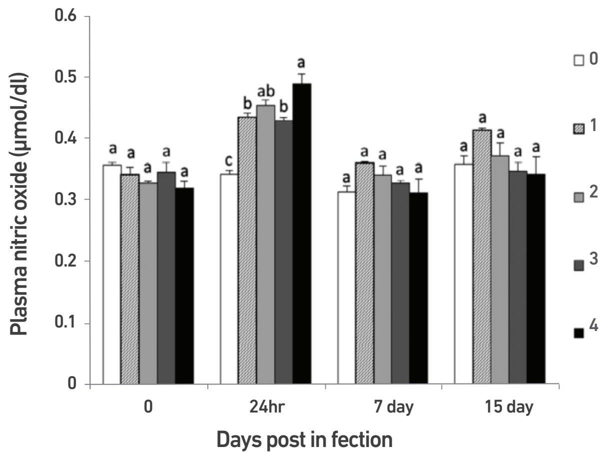
Figure 4. Percent cumulative mortality of fingerling channel catfish fed incremental levels of L-arginine (% of diet) for 2 weeks and subsequently exposed to Edwardsiella ictaluri.

Vianna et al. (2020) also evaluated the effects dietary arginine on resistance of tilapia fingerlings to Streptococcus agalactiae. After 30 days feeding trial with 5 graded levels of arginine (1.39%, 1.76%, 1.97%, 2.18%, and 2.39% of diet) for the final weighting and blood sampling, fish were inoculated with a suspension containing Streptococcus agalactiae strain S13 and returned to their respective tanks. During the challenge test, fish mortalities were counted, and blood samples were collected as described in the previous section at 0 hr, 24 hr, 7 days and 15 days, and used to measure the parameters of respiratory outbreak of leucocytes and nitric oxide production. The study results shown that increasing levels of arginine in the diets decreased the mortality rates. Fish fed the dietary without arginine (1.39%) supplementation had the highest mortality (peak mortality) and lasted 15 days, and the group with the highest amount of arginine (2.39%) administration had the lowest mortality rate, and consequently the highest survival (Figure 5). In addition, the study also showed that an increase in the production of nitric oxide after 24 hr with arginine supplementation, with the 4% and 2% treatment showing better results (0.491 and 0.456 µmol/dl) (Figure 6), which presented a challenge to the immune system of the fish. The elevation of serum arginine in higher dietary levels of arginine probably contributed to higher production of nitric oxide, since the macrophages used arginine as a source of nitrogen for the synthesis of NO (Pohlenz et al., 2012; Zhang et al., 2014).



0 = 1.55% arginine; 1 = 1.76% arginine; 2 = 1.97% arginine; 3 = 2.18% arginine; 4 = 2.39% arginine, Vianna et al. (2020).

Figure 5. Survival rate of Nile tilapia alevin fed for 4 weeks with supplementation of increasing arginine levels in the diet at time 0 (preinoculation) and 24 hr after inoculation, 7 days and 15 days after inoculation with Streptococcus agalactiae.



Different letters denote a significant difference (p < .05) by the Tukey test between the treatments within the specific times. Values are expressed as mean ± SEM Vianna et al. (2020).

Figure 6. Nitric oxide production of Nile tilapia alevin fed for four weeks with supplementation of increasing levels of arginine in the diet at time 0 (preinoculation), 24 hr, 7 days and 15 days after inoculation with Streptococcus agalactiae.

Conclusion

From the above studies it is understood that, arginine is not only an essential amino acid in all fish species but also an important functional amino acid. In addition, arginine is a sole precursor for nitric oxide which has been shown to mediate the macrophage's killing of various microorganisms including parasites, fungi, bacteria, and viruses. Administrating the diets with arginine may constitute an effective means of increasing the immunocompetency, disease resistance as well as resistance against stress tolerance of fish and; therefore, minimizing the need to medicate fish during enteric septicemia epizootics. The dietary arginine, at the higher level required for optimal growth, enhanced survival of fish exposed to pathogen microorganism or under the stress condition.

REFERENCES

1. Adams, L.B., Hibbs, J.B., Taintor, R.R. and Krahenbuhl, J.L., 1990. Microbiostatic effect of murine-activated macrophages for Toxoplasma gondii. Role for synthesis of inorganic nitrogen oxides from L-arginine. The Journal of Immunology, 144(7), pp.2725-2729.
2. Akarid, K., Sinet, M., Desforges, B. and Gougerot-Pocidalo, M.A., 1995. Inhibitory effect of nitric oxide on the replication of a murine retrovirus in vitro and in vivo. Journal of Virology, 69(11), pp.7001-7005.
3. Ali, A., Hassan, D., Saleha, A.A., Siti, K.B. and Milud, A., 2010. Streptococcus agalactiae the etiological agent of mass mortality in farmed red tilapia (Oreochromis sp.). Journal of Animal and Veterinary Advances, 9(20), pp.2640-2646.
4. Arias, M., Rojas, M., Zabaleta, J., Rodríguez, J.I., París, S.C., Barrera, L.F. and García, L.F., 1997. Inhibition of virulent Mycobacterium tuberculosis by Bcg r and Bcg s macrophages correlates with nitric oxide production. Journal of Infectious Diseases, 176(6), pp.1552-1558.
5. Barbul, A., 1995. The use of arginine in clinical practice. Amino acid metabolism and therapy in health and nutritional disease., pp.361-372.
6. Baldwin, T.J. and Newton, J.C., 1993. Pathogenesis of enteric septicemia of channel catfish, caused by Edwardsiella ictaluri: bacteriologic and light and electron microscopic findings. Journal of aquatic animal health, 5(3), pp.189-198.
7. Brittenden, J., Heys, S.D., Ross, J., Park, K.G.M. and Eremin, O., 1994. Nutritional pharmacology: effects of L-arginine on host defences, response to trauma and tumour growth. Clinical Science, 86(2), pp.123-132.
8. Buentello, J.A. and Gatlin III, D.M., 2001. Effects of elevated dietary arginine on resistance of channel catfish to exposure to Edwardsiella ictaluri. Journal of Aquatic Animal Health, 13(3), pp.194-201.
9. Chen, Q., Zhao, H., Huang, Y., Cao, J., Wang, G., Sun, Y. and Li, Y., 2016. Effects of dietary arginine levels on growth performance, body composition, serum biochemical indices and resistance ability against ammonia-nitrogen stress in juvenile yellow catfish (Pelteobagrus fulvidraco). Animal Nutrition, 2(3), pp.204-210.
10. Chen, G., Liu, Y., Jiang, J., Jiang, W., Kuang, S., Tang, L., Tang, W., Zhang, Y.A., Zhou, X. and Feng, L., 2015. Effect of dietary arginine on the immune response and gene expression in head kidney and spleen following infection of Jian carp with Aeromonas hydrophila. Fish & Shellfish Immunology, 44(1), pp.195-202.
11. Cheng, Z., Gatlin III, D.M. and Buentello, A., 2012. Dietary supplementation of arginine and/or glutamine influences growth performance, immune responses and intestinal morphology of hybrid striped bass (Morone chrysops× Morone saxatilis). Aquaculture, 362, pp.39-43.
12. Cheng, Z., Buentello, A. and Gatlin III, D.M., 2011. Effects of dietary arginine and glutamine on growth performance, immune responses and intestinal structure of red drum, Sciaenops ocellatus. Aquaculture, 319(1-2), pp.247-252.
13. Costas, B., Conceição, L.E., Dias, J., Novoa, B., Figueras, A. and Afonso, A., 2011. Dietary arginine and repeated handling increase disease resistance and modulate innate immune mechanisms of Senegalese sole (Solea senegalensis Kaup, 1858). Fish & shellfish immunology, 31(6), pp.838-847.
14. De Groote, M.A. and Fang, F.C., 1995. NO inhibitions: antimicrobial properties of nitric oxide. Clinical Infectious Diseases, 21(Supplement_2), pp.S162-S165.
15. Hawke, J.P., Mcwhorter, A.C., Steigerwalt, A.G. and Brenner, D.J., 1981. Edwardsiella ictaluri sp. nov., the causative agent of enteric septicemia of catfish. International Journal of Systematic and Evolutionary Microbiology, 31(4), pp.396-400.
16. Hibbs, J.B., Taintor, R.R. and Vavrin, Z., 1987. Macrophage cytotoxicity: role for L-arginine deiminase and imino nitrogen oxidation to nitrite. Science, 235(4787), pp.473-476.
17. Khan, D., 2019. Metabolic & performance benefits of L-Arginine over guanidinoacetic acid. http://www.positiveaction.info/pdfs/articles/pp27_5p17.pdf
18. Granger, D.L., Hibbs, J.B., Perfect, J.R. and Durack, D.T., 1988. Specific amino acid (L-arginine) requirement for the microbiostatic activity of murine macrophages. The Journal of clinical investigation, 81(4), pp.1129-1136.
19. Nakano, T., 2007. Microorganisms. In Dietary Supplements for the Health and Quality of Cultured Fish (pp. 86-108). CABI Publishing.
20. Narita, I., Border, W.A., Ketteler, M.A.R.K.U.S., Ruoslahti, E. and Noble, N.A., 1995. L-arginine may mediate the therapeutic effects of low protein diets. Proceedings of the National Academy of Sciences, 92(10), pp.4552-4556.
21. MacMillan, J. 1985. Infectious disease. Pages 4342441 in C. S. Tucker, editor. Channel catfish culture. Developments in aquaculture and fisheries science, 15. Elsevier Publishers, New York
22. Mian, G.F., Godoy, D.T., Leal, C.A.G., Yuhara, T.Y., Costa, G.M. and Figueiredo, H.C.P., 2009. Aspects of the natural history and virulence of S. agalactiae infection in Nile tilapia. Veterinary microbiology, 136(1-2), pp.180-183.
23. Moinard, C., Chauveau, B., Walrand, S., Felgines, C., Chassagne, J., Caldefie, F., Cynober, L.A. and Vasson, M.P., 1999. Phagocyte functions in stressed rats: comparison of modulation by glutamine, arginine and ornithine 2-oxoglutarate. Clinical Science, 97(1), pp.59-65.
24. Lall, S., 1993. Role of micronutrients in immune response and disease resistance in fish. In Fish Nutrition in Practice. IV International Symposium on Fish Nutrition and Feeding, INRA, Paris (pp. 101-118).
25. Lancaster Jr, J.R., 1992. Nitric oxide in cells. American Scientist, 80(3), pp.248-259.
26. Leone, A.M., Palmer, R.M., Knowles, R.G., Francis, P.L., Ashton, D.S. and Moncada, S., 1991. Constitutive and inducible nitric oxide synthases incorporate molecular oxygen into both nitric oxide and citrulline. Journal of Biological Chemistry, 266(35), pp.23790-23795.
27. Li, Y.W., Liu, L., Huang, P.R., Fang, W., Luo, Z.P., Peng, H.L., Wang, Y.X. and Li, A.X., 2014. Chronic streptococcosis in N ile tilapia, O reochromis niloticus (L.), caused by S treptococcus agalactiae. Journal of Fish Diseases, 37(8), pp.757-763.
28. Lin, H., Tan, X., Zhou, C., Niu, J., Xia, D., Huang, Z., Wang, J. and Wang, Y., 2015. Effect of dietary arginine levels on the growth performance, feed utilization, non-specific immune response and disease resistance of juvenile golden pompano Trachinotus ovatus. Aquaculture, 437,

pp.382-389.
29. Plumb, J. A. 1994. Enteric septicemia of catfish. Pages 1422148 in Health and maintenance of cultured fish, Principal microbial diseases, part three: bacterial diseases. CRC Press, Boca Raton, Florida.
30. Pohlenz, C., Buentello, A., Mwangi, W. and Gatlin III, D.M., 2012. Arginine and glutamine supplementation to culture media improves the performance of various channel catfish immune cells. Fish & Shellfish Immunology, 32(5), pp.762-768.
31. Rahnama, B., 2010. Supplementing a commercial diet for atlantic salmon (Salmo salar L.) with arginine, glutamate or tetradecylthioacetic acid (TTA). impact on production efficiency, slaughter parameters and flesh quality (Master's thesis).
32. Reyes, A.A., Karl, I.E. and Klahr, S.A.U.L., 1994. Role of arginine in health and in renal disease. American Journal of Physiology-Renal Physiology, 267(3), pp.F331-F346.
33. Robinson, L.E., Bussiere , F.I., Le Boucher, J., Farges, M.C., Cynober, L.A., Field, C.J. and Baracos, V.E., 1999. Amino acid nutrition and immune function in tumour-bearing rats: a comparison of glutamine-, arginine-and ornithine 2-oxoglutarate-supplemented diets. Clinical Science, 97(6), pp.657-669.
34. Sahu, M.K., Swarnakumar, N.S., Sivakumar, K., Thangaradjou, T. and Kannan, L., 2008. Probiotics in aquaculture: importance and future perspectives. Indian journal of microbiology, 48(3), pp.299-308.
35. Soto, E., Wang, R., Wiles, J., Baumgartner, W., Green, C., Plumb, J. and Hawke, J., 2015. Characterization of isolates of Streptococcus agalactiae from diseased farmed and wild marine fish from the US Gulf Coast, Latin America, and Thailand. Journal of aquatic animal health, 27(2), pp.123-134.
36. Vianna, R.A., Chideroli, R.T., da Costa, A.R., Ribeiro Filho, O.P., de Oliveira, L.L., Donzele, J.L., Lanna, E.A.T., Gonçalves, D.D. and Pereira, U.D.P., 2020. Effect of experimental arginine supplementation on the growth, immunity and resistance of tilapia fingerlings to Streptococcus agalactiae. Aquaculture Research, 51(3), pp.1276-1283.
37. Yang, Q., Liu, J.X., Wang, K.Y., Liu, T., Zhu, L., He, S.Y., Geng, Y., Chen, D.F., Huang, X.L. and Ou-yang, P., 2018. Evaluation of immunogenicity and protective efficacy of the elongation factor Tu against Streptococcus agalactiae in tilapia. Aquaculture, 492, pp.184-189.
38. Zhang, W., Sun, S., Ge, X., Zhu, J., Li, B., Miao, L., Xia, S., Zhang, Q. and Jiang, X., 2015. Acute effects of ammonia exposure on histopathology of gill, liver and kidney in juvenile Megalobrama amblycephala and the post-exposure recovery. Journal of Fisheries of China, 39(2), pp.233-244.
39. Zhang, C.N., Li, X.F., Jiang, G.Z., Zhang, D.D., Tian, H.Y., Li, J.Y. and Liu, W.B., 2014. Effects of dietary fructooligosaccharide levels and feeding modes on growth, immune responses, antioxidant capability and disease resistance of blunt snout bream (Megalobrama amblycephala). Fish & shellfish immunology, 41(2), pp.560-569.
40. Zhi, L., Yong-jian, L., Kang-sen, M. and Li-xia, T., 2004. Advance in researches on arginine requirement for fish: a review. Shuichan Xuebao, 28(04), pp.450-459.
41. Zhou, Q., Jin, M., Elmada, Z.C., Liang, X. and Mai, K., 2015. Growth, immune response and resistance to Aeromonas hydrophila of juvenile yellow catfish, Pelteobagrus fulvidraco, fed diets with different arginine levels. Aquaculture, 437, pp.84-91.
42. Wu, M., Wu, X., Lu, S., Gao, Y., Yao, W., Li, X., Dong, Y. and Jin, Z., 2018. Dietary arginine affects growth, gut morphology, oxidation resistance and immunity of hybrid grouper (Epinephelus fuscoguttatus♀× Epinephelus lanceolatus♂) juveniles. British Journal of Nutrition, 120(3), pp.269-282.
43. Wu, G., Flynn, N.E., Flynn, S.P., Jolly, C.A. and Davis, P.K., 1999. Dietary protein or arginine deficiency impairs constitutive and inducible nitric oxide synthesis by young rats. The Journal of nutrition, 129(7), pp.1347-1354.