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Review Article

Unraveling the Immunomodulatory Potential of *Cyprinus carpio*

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ABSTRACT

Recent advances in animal studies for exploring dominant behaviors and human disease models have recognized fish as popular vertebrate models having comparable basic physiology and molecular biology. Despite the divergence from mammals about 400 years ago, the fish model system has achieved substantial acceptance owing to its homogeneity and ease of availability. The Common Carp (*Cyprinus carpio*) is the world's most consumed and bred freshwater fish, with significant size and high fecundity, which allows superior analysis of the biological processes and to explore the effect of genetics and transgenetic endeavors. Because of its extensive aquaculture, the common carp has also become vulnerable to diseases and infections under the influence of certain inducers. To mitigate these diseases, a recent approach has been the use of immunomodulators/immunostimulants that influence and strengthen the host's immune response. These agents can be biological (certain bacteria) or synthesized chemicals that stimulate the innate immunity to act via cellular or humoral pathways. The data was collected by referring to various databases such as Wiley, Elsevier, PubMed, and Web of Science sources. The paper presents a comprehensive review of the various agents used so far for immunomodulation in the common carp. Subsequently, the role of certain inflammatory mediators like cytokines, tumor necrosis factors, interleukins, and nitric oxide synthase has been discussed in the light of both human and fish research. This review article highlights the utility of common carp as an immunomodulatory model, besides bringing to attention the potential pathways and molecular targets that may be used for developing alternate therapies for common diseases. Furthermore, it also aims to suggest a potential collaboration between marine and pharmaceutical researchers that can help to enlighten the path in the surge of new drug candidates in the future.

Keywords: Immune system, Cytokine, Cyprinus carpio, Common Carp, Immunomodulatory model, TNF-α, IL-1β, NOS.

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INTRODUCTION

For a long time, animal studies to explore the human disease models have been performed on mammals, especially rodents that closely resemble the human body, both morphologically as well as physiologically. Over the years, there has been a shift to using fish as vertebrates to get an idea of the complex phenomenon that underlies human diseases and the response of the human body.[1] Although evolution-wise, Pisces diverged from mammals and other classes of vertebrate animals about 400 million years ago, the basic body morphology and molecular physiology are comparable to their mammalian counterparts. Thus, the suitability of fishes, particularly the zebrafish and the common carp, is commonly used in research on human diseases.[2]

For biomedical purposes, fish farming is advantageous as they are small creatures with a high fertility rate/high fecundity and shorter lifespan, thus requiring less monetary and logistic efforts for breeding.[1] Also, the availability of a larger sample with high turnover allows a superior analysis of the biological processes as well as exploring the effect of genetics and transgenic endeavors. Furthermore, it is possible to employ high-throughput techniques of whole-genome mutagenesis or chemical-library drug screens on a readily available large Piscean sample, ensuring versatility in research. Likewise, its recent popularity and suitability for biomedical research have promoted the use of fish as a resourceful vertebrate model to investigate dominant behavior in animals.[3] Regarding the ability to display ample traits and features of dominance-behavior in animal hierarchical studies, fishes are comparable to the usual sample of chickens, who are extensively studied. Additionally, it is easier to maintain fish in the laboratory than chickens and they display the dominance-behavior of aggression well enough, both in the lab and the wild.

Consequently, zebrafish are extensively used for animal studies as they are low-maintenance, highly fertile, and can be used for a variety of experiments as they have significant genomic similarities with humans. Their mutant varieties are easily available and have lesser legal restrictions associated with their use for biomedical purposes.[4,5] They are widely used in toxicology studies owing to their size and ease of availability. Other species that are used for similar reasons are the Japanese medaka and the common guppy. The Common Carp, a native Asian fish originally found in China and naturally inhabiting up to Turkistan and east of the Caspian Sea, belongs to the same cyprinid family as zebrafish, has a larger size than the zebrafish, and is the most widely consumed and cultivated freshwater fish.[6–9] Both these fish can be used in ecological research studying the aquatic environments besides their importance as valuable contributors to monitoring aquatic pollution. However, despite their resourcefulness in physiological, biochemical, and molecular studies owing to the feasibility in laboratory settings, the distribution of fish cannot be compared to the traditional small rodents used for human disease models.[4,5]

Due to the widespread consumption and the consequent growth of the aquaculture industry, the common carp has become vulnerable to disease and infection. The common carp has genes that belong to the MEF-2 group, which are similar to the MEFA2 genes found in vertebrates.[10,11] To mitigate these problems and prevent the spread within the aquatic environment as well as to humans, various immunostimulants have been introduced to improve the common carp's immunity and study their effect on human diseases and also study its use in developing vaccines.[12,13] Aquaculture has become an increasingly important field over the last decades as more fish models are being explored in various neurological, toxicological, and immunological studies. However, with increasing popularity, the vulnerability of aquacultures to a variety of infections and diseases has also risen. These diseases and infections are induced by certain inducers which modify the host's immune system. To counter these inducers, many immunostimulants have been widely developed and used for fish models. Common carp is a prominent fish model used for immunomodulation studies, and this review gives a comprehensive overview of the potential of common carp as a utility model for immunomodulation. Figure 1 provides understanding related to the utility of the common carp model. We have also reviewed various inducers that affect the immune response in common carp, besides describing its cytokine and immune system. It is believed that a deeper and more versed understanding of the importance of various cytokines in fish will help pharmaceutical researchers in the development of novel and potent immunomodulatory agents. This review article highlights the utility of common carp as an immunomodulatory model, besides bringing to attention the potential pathways and molecular targets that may be used for developing alternate therapies for common diseases.

METHODS

In this review, the literature search was undertaken by exploring databases including PubMed, Scopus, Wiley, Elsevier, Google Scholar, Google Patents, and Web of Science which were published up to January 2021 were collected. The compiled review contains 49 references which include patents along with a review as well as research articles, case studies, and book chapters. The literature was searched using keywords such as Immune system, cytokine, *Cyprinus carpio*, Common Carp, immunomodulatory model, TNF-α, IL-1β, NOS. Figure 2 depicts the flowchart of the methodology used in this article.

Cyprinus carpio as an immunomodulatory model

Due to their sizeable consumption and culture, common carp farming, one of the fastest-growing fish-farming industries globally, faces the dangers of disease and infection. The first sample of Common Carp / Common Safar / American Rohu (*Cyprinus carpio*, family: *Cyprinidae*), a native Asian fish, in India was received from Sri Lanka in 1930 as the subspecies Mirror Carp and was kept in the Utakmand lake, Ooty, Tamil Nadu.[14,15] In Japan, its ornamental form, known as the *koi* is still

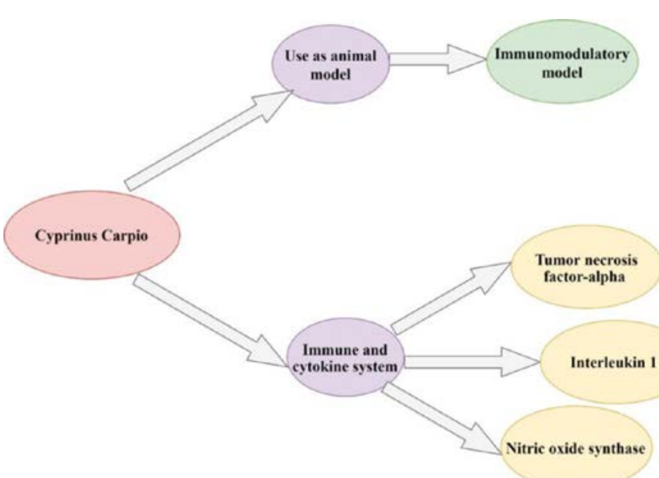

Figure 1: Role of *Cyprinus carpio* in immunomodulatory mechanisms.

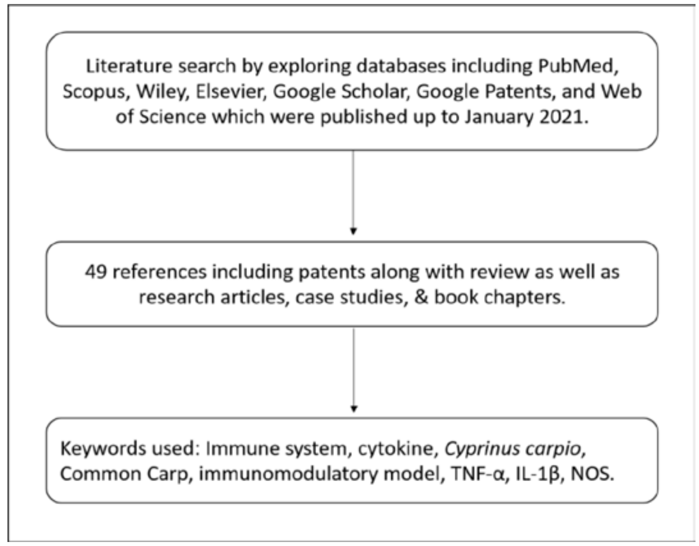

Figure 2: Methodology flow chart of *Cyprinus carpio*.

considered as a symbol of are still symbolic of valor and vitality. This native Asian fish spread across the globe through humans and can now be found on all continents except Antarctica and is bred in both ponds and captive fisheries. *C. carpio* farming accounts for about 11% of the world's total freshwater aquaculture owing to its high reproduction rate, of which 90% is done in the Asian countries, and is an important aquatic species due to its role in checking aquatic pollution, as well as its ability to withstand extreme environmental conditions.[14–16]

Different aquaculture systems adapt their food habits depending on the input availability of food and nutrients, resulting in changed food niches and varying food selectivity. Similar changes were seen in common carp populations of Asia and Europe.[8] Their behavior related to food preference and food niches changed as the European wild carp populations significantly declined since the last glacial period, while the Asian population remained unaffected. This sparing of the Asian carp could be attributed to a separation of the European and Asian clades much before the glacial melting.[17]

Furthermore, the common carp possesses multiple myocyte enhancer factor 2 (MEF2) genes, which are common in belonging to the MADS-box superfamily of eukaryotic transcription factors.[9] The vertebrates also contain genes from the MADS-box superfamily, in the form of 4 distinct subfamilies, namely MEF2 - A, B, C, and D.[18]

Immunostimulants, which aim to promote both specific and non-specific immunity in the fish culture, are the potential agents to mitigate these dangers. Many studies have been performed that evaluate the efficacy of such immunomodulators/ immunostimulants using herbal feed supplemented with the drugs and no gross side effects were seen in the common carp. Further, it was observed that such feeds improved immunocompetence and disease resistance, besides serving as effective dietary nutrient resources to ensure maximum growth.[10,11]

Also, multiple natural and synthetic potential immunomodulatory agents, which influence the innate immunity in the common carp, have been used and their effect studied, namely *Aeromonas hydrophila*, *Escherichia coli, Pseudomonas fluorescens*, *Aeromonas salmonicida*, *Edwardsiellatarda*, *Vibrio parahaemolyticus*, ammonium chloride, cadmium chloride.[19-26] The various hemato-immunological parameters of innate immunity evaluated for these agents include total serum protein albumin, globulin, albumin/globulin (AG) Ratio, serum lysozyme activity, serum bactericidal activity, plasma cortisol concentration, differential white-blood-cell count, neutrophil activity, growth parameters, mortality rates, respiratory burst antibody responses, and phagocytosis and macrophage activating factors.[27,28] Table 1 presents an overview of the various immunomodulatory agents used and their target tissue.

Role of immune and cytokine system in *Cyprinus carpio*

In a common carp embryo, the skin mucosa forms the primary defense as a part of the innate immune system through mucin secretion, antimicrobial peptides, and enzymatic activity. A secondary barrier is formed through macrophages, granulocytes, and humoral factors against the attacking pathogens. Lastly, the immune system also can both adapt and respond to specific known antigens, which are identified as 'non-self' through memory. This adaptive immune system in common carp starts developing by the 4th day after fertilization and becomes fully functional by the first 2 months.[29]

Unlike the higher vertebrates, the Piscean immune responses have not been defined completely and several important immunity genes are homologous to mammals identified in many species of fish. These findings suggest that although the two classes of vertebrates may have diverged in evolution, the basic system of immune responses is comparable in both. The discovery of α and β T-cell receptor genes in fish indicates that mammalian-like T-helper cells – Th1, Th2, and their regulatory subset Treg may also be present in fish. Further, antibodies found in teleosts are crucial in executing an immune response and IgM-immunoglobulin is an important component of specific humoral response seen in teleosts against numerous invading pathogens.[30]

Another important way of exerting the immune response is through cytokines, which are small glycoprotein mediators primarily responsible for the host's innate immunity. Produced primarily by macrophages and T-helper cells, cytokines can be categorized as interleukins (ILs), tumor necrosis factors (TNFs), transforming growth factors, chemokines, and interferons, based on their structural features. They act via cytokine receptors and have both pro-inflammatory and anti-inflammatory actions besides pathogen-killing activities. It has been reported that the TLR (Toll-like receptor) agonist initiates a non-specific immune response in common carp by evoking the cytokine-signaling pathway, which is further regulated through complex mechanisms involving intracellular TLR domain-containing adaptor molecules.[31] Notably, analyzing the expression profile of these cytokine-inducing genes and assessing their products involved in exerting the host's defense help to identify potential indirect immunological markers.[32] Many *in-vivo* and *in-vitro* studies have identified the common carp's genes that participate in the fish's immune response. These studies have recognized carp cytokines - interleukin-1 beta (IL1-β) and tumor necrosis factor-alpha (TNF-α), nitric oxide synthase (NOS), CXC-Chemokines and serum proteins complement factor-3, serum amyloid A, and alpha-2-macroglobulin responsible for the carp innate immunity.[33-35] The following sections discuss the role of some of these factors.

Table 1: Transcriptomics studies on fish after treatments with bacteria and chemicals.

Origin	Inducer	Tissue/Cell type	Infection route	References
Bacteria	*Aeromonas hydrophila*	Kidney, spleen	Intraperitoneal	43
	Aeromonas salmonicida	Thymus, kidney, spleen, gut-associated lymphoid tissue	Intraperitoneal	22,12
	Escherichia coli	Skin, blood, spleen	Intraperitoneal	44
	Pseudomonas fluorescens	Heart, kidneys, liver	Intraperitoneal	45
	Edwardsiellatarda	Liver, spleen	Intraperitoneal	46
	Vibrio parahaemolyticus	Kidney	Intraperitoneal	24
Chemicals	Ammonium chloride	Kidneys, liver	Intraperitoneal	25
	Cadmium chloride	Kidney	Intraperitoneal	47,48

Tumor necrosis factor-alpha (TNF-α)

TNF-α is a potent pro-inflammatory cytokine that is produced when inflammation occurs in response to viral, bacterial, and parasite infections. Factors with activity similar to the TNF have been found in fish, besides the recent evidence of heightened cell response in the form of leucocytic migration and increased phagocytic action of macrophages when the rainbow trout was administered and incubated with recombinant TNF proteins.[36] TNF-α is produced by a variety of cells including lymphocytes (T and B), macrophages/monocytes, and NK cells. Additionally, the gene responsible for TNF-α was identified and cloned from various Piscean species, namely the Japanese flounder, zebrafish, common carp, rock bream and grouper, and the seabream. Furthermore, a study exploring the effect of recombinant TNF-α at the molecular level in macrophages of rainbow trout has revealed that the expression of interleukins 1b, 8, and COX-2 was upregulated when treated with recombinant TNF-α.[37]

Interleukin 1 β (IL-1β)

Like TNF-α, Interleukin-1β is also a powerful pro-inflammatory cytokine that is released in the case of both infections and injury. Notably, the release of IL-1β is induced neither by a signaling pathway nor triggered by the conventional protein-secretion, rather, non-conventional pathways are responsible for its release.[38] The Interleukin-1 family is further composed of 11 subtypes with each having comparable or unique biological effects - IL-1α, IL-1β, IL-1Ra, IL-18, IL-33, IL-36α, IL-36β, IL-36γ, IL-36Ra IL-37, and IL-38. The IL-1β, a precursor to the 269-AA protein and processed by caspase-1, is activated in inflammasomes and encourages monocytic differentiation into the standard dendritic cells (DCs) and M1-like macrophages. Furthermore, it reinforces the process in which activated B- lymphocytes proliferate and differentiate into mature plasma cells. Consequently, raised serum IL-1β levels in humans and mice have been associated with an increased Th17-dominant immunopathology.[39]

It can be noted that cytokine IL-1 β is crucial for the host's innate immune and inflammatory responses and affects the regulation of the hypothalamus-pituitary-adrenal axis. However, studies exploring the association of cytokines and the activation of the hypothalamic-pituitary-inter-renal axis are insufficient to deduce conclusions. Still, there are reports which describe the effects of IL-1β on HPI-axis activity which further regulate and modify the gene expression of IL-1β and its receptors in conditions of acute stress.[40]

Nitric oxide synthase (NOS)

Nitric oxide is also a signaling molecule that is produced by 3 isoforms, namely neuronal NOS, endothelial NOS, and inducible NOS. Being the smallest-known signaling molecule, all NOS molecules bind closely with calmodulin, besides containing haem (Iron). The NOS molecule is produced in a variety of cells under the influence of lipopolysaccharides and cytokines. Furthermore, inducible NOS participates in the pathogenesis of a variety of inflammatory diseases as well as a septic shock.[41] Also, *ex-vivo* studies have shown that adding nitric oxide to thrombocytes isolated from healthy carp induced apoptosis, suggesting that nitric-oxide may have a role in mediating the activity of thrombocytes during an immune reaction in response to infection.[42]

CONCLUSION

This review highlights the importance of common carp as a potential utility model that can be used to study immunomodulation to counter diseases and infection. This paper will help to upsurge the interest in using common carp with the added advantage of bringing marine and pharmaceutical researchers together. This novel approach may promote the identification of new target entities in the immunomodulatory mechanism that can be used as an alternate mode of therapy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

IL: interleukin; **TNF-α:** Tumor necrosis factor-alpha; **NOS:** Nitric oxide synthase; **AG:** albumin/globulin.

REFERENCES

1. Schartl M. Beyond the zebrafish: Diverse fish species for modeling human disease. Dis Model Mech. 2014;7(2):181-92. doi: 10.1242/dmm.012245, PMID 24271780.
2. Harris MP, Henke K, Hawkins MB, Witten PE. Fish is Fish: The use of experimental model species to reveal causes of skeletal diversity in evolution and disease. J Appl Ichthyol [internet]. 2014;30(4):616-29. doi: 10.1111/jai.12533, PMID 25221374.
3. Chase ID, Seitz K. Self-structuring properties of dominance hierarchies a new perspective [internet]. Adv Genet. 2011;75:51-81. doi: 10.1016/B978-0-12-380858-5.00001-0, PMID 22078477.
4. Fox JG, Anderson LC, Loew FM, Quimby FW. Laboratory animal medicine. Academic press; 2002.
5. Ali S, Champagne DL, Spaink HP, Richardson MK. Zebrafish embryos and larvae: A new generation of disease models and drug screens. Birth Defects Res C Embryo Today. 2011;93(2):115-33. doi: 10.1002/bdrc.20206, PMID 21671352.
6. Carp C, Fisherman Welfare and Fisheries Development Department, Government of Madhya Pradesh [internet] [cited Nov 24 2021]. Available from: http://www.mpfisheries.gov.in/common-carp.
7. *Cyprinus carpio* (common carp) [internet] [cited Nov 24 2021]. Available from: https://www.cabi.org/isc/datasheet/17522.
8. Rahman MM, Kadowaki S, Balcombe SR, Wahab MA. Common carp (*Cyprinus carpio* L.) alters its feeding niche in response to changing food resources: direct observations in simulated ponds. Ecol Res. 2009;25:303-9. doi: 10.1007/s11284-009-0657-7.
9. He M, Zhou D, Ding NZ, Teng CB, Yan XC, Liang Y. Common carp mef2 genes: Evolution and expression. Genes (Basel). 2019;10(8):588. doi: 10.3390/genes10080588, PMID 31374988.
10. Soltanian S, Fereidouni MS. Effect of Henna (*Lawsonia inermis*) extract on the immunity and survival of common carp, *Cyprinus carpio* infected with Aeromonas hydrophila. Int Aquat Res. 2016;8:247-61. doi: 10.1007/s40071-016-0141-2.
11. Pratheepa V, Ramesh S, Sukumaran N. Immunomodulatory effect of *Aegle marmelos* leaf extract on freshwater fish *Cyprinus carpio* infected by bacterial pathogen *Aeromonas hydrophila*. Pharm Biol. 2010;48(11):1224-39. doi: 10.3109/13880201003713598, PMID 20818950.
12. Vetvicka V, Vannucci L, Sima P. The effects of – glucan on fish immunity. N Am J Med Sci. 2013;5(10):580-8. doi: 10.4103/1947-2714.120792, PMID 24350069. Available from: /pmc/articles/PMC3842698. Same as Reference #44
13. Ma J, Bruce TJ, Jones EM, Cain KD. A review of fish vaccine development strategies: Conventional methods and modern biotechnological approaches. Microorganisms. 2019;7(11). doi: 10.3390/microorganisms7110569, PMID 31744151. Available from: /pmc/articles/PMC6920890.
14. Costa Pierce BA, Moreau J, Pullin RSV. New introductions of common carp and their impact on indigenous species in sub-Saharan Africa. Discov Innov. 1993;5:211-21.
15. B n rescu P, Coad BW. Cyprinids of Eurasia. Cyprinid Fishes. 1991:127-55.
16. Welcomme R. A history of international introductions of inland aquatic species; 2013.
17. Yuan Z, Huang W, Liu S, Xu P, Dunham R, Liu Z. Historical demography of common carp estimated from individuals collected from various parts of the world using the pairwise sequentially markovian coalescent approach. Genetica. 2018;146(2):235-41. doi: 10.1007/s10709-017-0006-7, PMID 29299799.
18. Wu W, De Folter S, Shen X, Zhang W, Tao S. Vertebrate paralogous MEF2 genes: Origin, conservation, and evolution. PLOS ONE. 2011;6(3):e17334. doi: 10.1371/journal.pone.0017334, PMID 21394201.
19. Zhao XL, Jin ZH, DI GL, Li L, Kong XH. Molecular characteristics, pathogenicity and medication regimen of *Aeromonas hydrophila* isolated from common carp (*Cyprinus carpio* L.). J Vet Med Sci. 2019/08/12;81(12):1769-75. doi: 10.1292/jvms.19-0025, PMID 31406034.
20. Nguyen TM, Mandiki SNM, Salomon JMAJ, Baruti JB, Thi NTT, Nguyen TH, *et al*. Pro- and anti-inflammatory responses of common carp *Cyprinus carpio* head kidney leukocytes to E.coli LPS as modified by different dietary plant oils. Dev Comp Immunol. 2021;114:103828. doi: 10.1016/j.dci.2020.103828.
21. Pekala-Safinska A. Contemporary threats of bacterial infections in freshwater fish. J Vet Res. 2018;62(3):261-7. doi: 10.2478/jvetres-2018-0037, PMID 30584602.
22. Nakayama K, Yamashita R, Kitamura SI. Use of common carp (*Cyprinus carpio*) and *Aeromonas salmonicida* for detection of immunomodulatory effects of chemicals on fish. Mar Pollut Bull. 2017;124(2):710-3. doi: 10.1016/j.marpolbul.2016.12.060, PMID 28063699.
23. Sahoo PK, Mukherjee SC. The effect of dietary immunomodulation upon *Edwardsiella tarda* vaccination in healthy and immunocompromised Indian major carp (Labeo rohita). Fish Shellfish Immunol. 2002;12(1):1-16. doi: 10.1006/fsim.2001.0349, PMID 11866127.
24. M S, B D. Immunostimulatory Effects of Polysaccharide Compound from Seaweed Kappaphycus alvarezii on Asian seabass (Lates calcarifer) and itâ s Resistance against Vibrio parahaemolyticus. J Mar. Biol Oceanogr. 2015;04. doi: 10.4172/2324-8661.1000144.
25. Taheri Mirghaed A, Fayaz S, Hoseini SM. Dietary 1,8-cinoele affects serum enzymatic activities and immunological characteristics in common carp (*Cyprinus carpio*) exposed to ambient ammonia. Aquac Res. 2018;50:146-53. doi: 10.1111/are.13877.
26. Zhang Z, Zheng Z, Cai J, Liu Q, Yang J, Gong Y, *et al*. Effect of cadmium on oxidative stress and immune function of common carp (*Cyprinus carpio* L.) by transcriptome analysis. Aquat Toxicol. 2017;192:171-7. doi: 10.1016/j.aquatox.2017.09.022, PMID 28963925.
27. Maqsood S, Samoon M, Singh P. Immunomodulatory and growth promoting effect of dietary levamisole in *Cyprinus carpio* fingerlings against the challenge of Aeromonas hydrophila. Turk J Fish Aquat Sci. 2009.
28. Gopalakannan A, Arul V. Immunomodulatory effects of dietary intake of chitin, chitosan and levamisole on the immune system of *Cyprinus carpio* and control of Aeromonas hydrophila infection in ponds. Aquaculture. 2006;255:179-87. doi: 10.1016/j.aquaculture.2006.01.012.
29. Huttenhuis HBT, Taverne-Thiele AJ, Grou CPO, Bergsma J, Saeij JPJ, Nakayasu C, Rombout JH. Ontogeny of the common carp (*Cyprinus carpio* L.) innate immune system. Dev Comp Immunol. 2006;30(6):557-74. doi: 10.1016/j.dci.2005.08.001, PMID 16406121.
30. Reyes-Cerpa S, Maisey K, Reyes-Lpez F, Toro-Ascuy D, Mara A, Imarai M. Fish cytokines and immune response [internet]. New Advances and Contributions to Fish Biology. doi: 10.5772/53504; 2012. InTech [cited 24/3/2022].
31. Kany S, Vollrath JT, Relja B. Cytokines in inflammatory disease. Int J Mol Sci. 2019;20(23). doi: 10.3390/ijms20236008, PMID 31795299. Available from: /pmc/articles/PMC6929211.
32. Biswas G, Korenaga H, Takayama H, Kono T, Shimokawa H, Sakai M. Cytokine responses in the common carp, *Cyprinus carpio* L. treated with baker's yeast extract. Aquaculture. 2012;356-357:169-75. doi: 10.1016/j.aquaculture.2012.05.019.
33. Saeij JPJ, De Vries BJd, Wiegertjes GF. The immune response of carp to *Trypanoplasma borreli*: Kinetics of immune gene expression and polyclonal lymphocyte activation. Dev Comp Immunol. 2003;27(10):859-74. doi: 10.1016/s0145-305x(03)00083-1, PMID 12880636.
34. Saeij JPJ, VAN Muiswinkel WB, Groeneveld A, Wiegertjes GF. Immune modulation by fish kinetoplastid parasites: A role for nitric oxide. Parasitology. 2002;124(1):77-86. doi: 10.1017/s0031182001008915, PMID 11817378.
35. Fujiki K, Shin DH, Nakao M, Yano T. Molecular cloning and expression analysis of carp (*Cyprinus carpio*) interleukin-1 beta, high affinity immunoglobulin E Fc receptor gamma subunit and serum amyloid A. Fish Shellfish Immunol. 2000;10(3):229-42. doi: 10.1006/fsim.1999.0253, PMID 10938736.
36. Savan R, Sakai M. Presence of multiple isoforms of TNF alpha in carp (*Cyprinus carpio* L.): genomic and expression analysis. Fish Shellfish Immunol. 2004;17(1):87-94. doi: 10.1016/j.fsi.2003.11.001, PMID 15145420.
37. Zhang A, Chen D, Wei H, Du L, Zhao T, Wang X, *et al*. Functional characterization of TNF-α in grass carp head kidney leukocytes: induction and involvement in the regulation of NF-κB signaling. Fish Shellfish Immunol. 2012;33(5):1123-32. doi: 10.1016/j.fsi.2012.08.029, PMID 22981915.
38. Lopez-Castejon G, Brough D. Understanding the mechanism of IL-1β secretion. Cytokine Growth Factor Rev. 2011/10/22;22(4):189-95. doi: 10.1016/j.cytogfr.2011.10.001, PMID 22019906.

39. Kaneko N, Kurata M, Yamamoto T, Morikawa S, Masumoto J. The role of interleukin-1 in general pathology. Inflamm Regen. 2019;39:12. doi: 10.1186/s41232-019-0101-5, PMID 31182982.

40. Metz JR, Huising MO, Leon K, Verburg-van Kemenade BML, Flik G. Central and peripheral interleukin-1beta and interleukin-1 receptor I expression and their role in the acute stress response of common carp, Cyprinus carpio L J Endocrinol. 2006;191(1):25-35. doi: 10.1677/joe.1.06640, PMID 17065386.

41. Förstermann U, Sessa WC. Nitric oxide synthases: Regulation and function. Eur Heart J. 2012;33(7):829-37, 837a. doi: 10.1093/eurheartj/ehr304, PMID 21890489.

42. Fink IR, Ribeiro CMS, Forlenza M, Taverne-Thiele A, Rombout JHWM, Savelkoul HFJ, *et al*. Immune-relevant thrombocytes of common carp undergo parasite-induced nitric oxide-mediated apoptosis. Dev Comp Immunol. 2015;50(2):146-54. doi: 10.1016/j.dci.2015.02.008, PMID 25681740.

43. Shahi N, Ardó L, Fazekas G, Gócza E, Kumar S, Rèvèsz N, *et al*. Immunogene expression in head kidney and spleen of common carp (*Cyprinus carpio* L.) following thermal stress and challenge with Gram-negative bacterium, Aeromonas hydrophila. Aquac Int. 2018;26:727-41. doi: 10.1007/s10499-018-0250-6.

44. Hang BT, Milla S, Gillardin V, Phuong NT, Kestemont P. *In vivo* effects of *Escherichia coli* lipopolysaccharide on regulation of immune response and protein expression in striped catfish (*Pangasianodon hypophthalmus*). Fish Shellfish Immunol. 2013;34(1):339-47. doi: 10.1016/j.fsi.2012.11.025, PMID 23207480.

45. Lamas Ferreiro JL, Álvarez Otero J, González González L, Novoa Lamazares L, Arca Blanco A, Bermúdez Sanjurjo JR, *et al*. Pseudomonas aeruginosa urinary tract infections in hospitalized patients: Mortality and prognostic factors. Plos one. 2017;12(5):e0178178. doi: 10.1371/journal.pone.0178178, PMID 28552972.

46. Bin PS, Aoki T, Jung TS. Pathogenesis of and strategies for preventing *Edwardsiella tarda* infection in fish. Vet Res. 2012;43:67.

47. Vincent S, Sumit R, Bavithra S, Victoria V, Akhila N, Prabha J. Metallothionein Induced Time Dependent histomorphometric Analysis of *Clarias gariepinus* Exposed to cadmium. Bull Environ Contam Toxicol. 2019;102(1):32-8. doi: 10.1007/s00128-018-2484-4, PMID 30406814.

48. Drag-Kozak E, Pawlica-Gosiewska D, Gawlik K, Socha M, Gosiewski G, Łuszczek-Trojnar E, *et al*. Cadmium-induced oxidative stress in Prussian carp (*Carassius gibelio* Bloch) hepatopancreas: ameliorating effect of melatonin. Environ Sci Pollut Res Int. 2019;26(12):12264-79. doi: 10.1007/s11356-019-04595-3, PMID 30835065. Available from: /pmc/articles/PMC6476825.

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