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Research on Antioxidant Function of Curcumin and Its Application in Fish Feed

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Abstract Curcumin is a yellow pigment derived from the rhizomes of ginger family plants, characterized by two phenolic hydroxyl groups and a β-diketone moiety in its structure. As a naturally occurring antioxidant, curcumin demonstrates significant antioxidant capacity through the formation of stable quinones during oxidative processes. The transcription factor Nrf2-mediated signaling pathway serves as a critical cellular antioxidant defense system, preserving cellular homeostasis through regulated antioxidant responses. This paper summarizes the antioxidative physiological roles of curcumin, its regulatory mechanism on reactive oxygen species (ROS) via the Nrf2 pathway, and its current applications in fish feed formulations.

Key words Curcumin, Nrf2 signaling pathway, Reactive oxygen species (ROS), Feed, Aquatic animal

0 Introduction

Curcumin is a yellow pigment extracted from the rhizome of plants in the Zingiberaceae family. It possesses a symmetrical molecular structure, with functional groups including two phenolic hydroxyl groups and a β-diketone moiety. Curcumin is a natural antioxidant, and quinones are the primary products generated during its antioxidant process[1]. Besides, curcumin exhibits various physiological effects, such as anti-inflammatory properties, immune enhancement, and lipid-lowering activity. As a crucial intracellular regulatory pathway for antioxidant stress, the transcription factor NF-E2-related factor 2 (Nrf2) maintains cellular health and homeostasis by regulating antioxidant responses. Understanding the antioxidant function and regulatory mechanisms of the Nrf2 signaling pathway is significant for a deeper comprehension of its role in the pathogenesis of diseases in aquatic animals. This review primarily summarizes the antioxidant physiological functions of curcumin, its mechanism of regulating reactive oxygen species (ROS) through the Nrf2 signaling pathway, and its application in fish feed.

1 Regulation of curcumin on ROS

1.1 ROS ROS are a class of highly reactive, oxygen-containing chemical species generated in living organisms. Possessing strong oxidizing capacity, they can oxidize a variety of substances. Common ROS include superoxide anion, hydrogen peroxide, and hydroxyl radical, which contain one or more unpaired electrons and exhibit high chemical reactivity. Under normal conditions, the production and scavenging of ROS in animal cells maintain a dynamic equilibrium. However, when animals are subjected to environmental or disease stress, this balance is disrupted, leading to a sharp increase in intracellular ROS levels[2-3]. In addition, given their high oxidative potential, an overabundance of ROS can compromise the integrity and function of essential biological macromolecules, including proteins, membrane lipids, and nucleic acids, leading to decreased productivity and retarded growth and development in animals[4]. Aquatic organisms are inherently prone to stress from their aqueous environment. Upon encountering pathological states or external challenges like heavy metals or heat stress, their cells can generate excessive ROS. This surge in ROS inflicts damage upon organs, tissues, and cells, thereby hindering critical processes such as development, growth, and immune function[5-6]. Furthermore, animal cells harbor multiple protective systems to mitigate and remove excess ROS. A primary line of defense involves antioxidant enzymes, notably catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and glutathione S-transferase (GST). This enzymatic defense is supplemented by antioxidant molecules like vitamin C and glutathione, which also actively participate in scavenging ROS. Collectively, these molecules and enzymes constitute the essential molecular machinery for reducing and eliminating ROS in animals[7].

1.2 Antioxidant properties of curcumin Peroxidation significantly influences the physiological and pathological processes in aquatic animals. ROS can disrupt the structure of intracellular molecules or directly impair the function of biological macromolecules through oxidative stress responses[8]. Curcumin possesses a unique chemical structure and is a typical hydrophobic polyphenolic compound. It contains phenolic hydroxyl and β-diketone groups, both of which can provide protons to block free radical chain reactions. Therefore, curcumin exerts its antioxidant effects primarily through these two functional groups[9].

Curcumin can directly scavenge free radicals and inhibit lipid peroxidation, thereby exerting its antioxidant effects. In the structural formula of curcumin, the phenolic group is essential for scavenging oxygen free radicals, the methylene group in the β-diketone moiety can donate protons, and the methoxy groups in the structure further enhance antioxidant activity. Compared with demethoxycurcumin and bisdemethoxycurcumin, curcumin exhibits

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superior antioxidant properties[10]. Trujillo *et al.*[11] found that the phenolic hydroxyl and methoxy groups of curcumin participate in scavenging ROS in animals, and curcumin can eliminate free radicals such as superoxide anion, hydroxyl radicals, and nitrogen dioxide *in vivo*.

When excessive ROS are generated within animal cells, the endogenous antioxidant system cannot eliminate them in a timely manner, leading to oxidative damage to intracellular biological macromolecules such as nucleic acids, proteins, and carbohydrates[12]. ROS that are not promptly cleared can induce physiological responses including lipid peroxidation, gene mutations, and protein denaturation, further damaging organelles such as lysosomes, peroxisomes, mitochondria, and ribosomes. Studies have found that curcumin not only scavenges ROS but also binds with metal ions such as manganese and copper to enhance the body's antioxidant capacity, thereby more effectively eliminating excess ROS[13-14]. In addition, curcumin can increase the activities of CAT, SOD, and GSH-Px, inhibit ROS production, and thus protect cells from oxidative stress damage[15-16].

1.3 Mechanism of curcumin regulating cell antioxidant function through Nrf2 signaling pathway

1.3.1 Nrf2. Nrf2 signaling pathway. Animals have evolved various mechanisms to prevent oxidative stress-induced cellular damage, with the transcription factor Nrf2 standing out as a critical regulator. Nrf2 exerts its protective effects by governing the antioxidant response element (ARE), a key promoter sequence that controls the expression of phase II detoxifying enzymes and antioxidant proteins. These include UDP-glucuronosyltransferase, heme oxygenase 1 (HO-1), glutathione S-transferase (GST), and NAD (P)H: quinone oxidoreductase (NQO). Through this pathway, Nrf2 orchestrates a comprehensive cellular defense against oxidative insult. The intracellular tumor suppressor protein Kelch-like ECH-associated protein 1 (Keap1) can form an Nrf2-Keap1 complex with Nrf2, thereby inhibiting Nrf2 activity[17] (Fig. 1). Only after the Nrf2-Keap1 complex dissociates can Nrf2 enter the nucleus and bind to ARE, activating the expression of downstream genes including GST, UDP-glucuronosyltransferase, HO-1, and quinone oxidoreductase (NQO)[17-18]. As an important antioxidant regulatory molecule in animal cells, Nrf2 exerts its antioxidant effects by regulating the gene expression levels of NAD (P) H: quinone oxidoreductase 1 (NQO1), HO-1, GST, and others[19].

1.3.2 Regulation of curcumin on Nrf2. Through the mediation of the antioxidant response element (ARE), Nrf2 plays an important role in inducing the expression levels of antioxidant genes. Curcumin can promote the gene expression level of Nrf2, thereby protecting cells from oxidative damage via Nrf2[20]. Nrf2 is a transcription factor that forms a complex with its cytoplasmic repressor Keap1 and is highly sensitive to redox reactions. As an adaptor protein between the Cullin 3 (Cul3) ubiquitin ligase complex and Nrf2, the N-terminal BTB domain of Keap1 can facilitate the ubiquitination of Nrf2, and ubiquitinated Nrf2 is subsequently degraded by the proteasome[21]. Studies have found that the cysteine residues of Keap1 can be modified by curcumin, leading to conformational changes in the Nrf2-Keap1 complex. This allows Nrf2 to dissociate from the complex and enter the nucleus. Once in the nucleus, Nrf2 forms a heterodimer with Maf proteins, binds to ARE in the nucleus, and induces the expression levels of phase II enzymes including HO-1, GST, and NQO[22].

Therefore, as an important antioxidant stress transcription factor in animal cells, Nrf2 exerts its antioxidant effects by regulating the expression levels of antioxidant-related genes. Under normal conditions in animal cells, Nrf2 forms a complex with Keap1 and remains in an inactive state. When animal cells are subjected to external oxidative stress, the Nrf2-Keap1 complex dissociates, allowing Nrf2 to enter the nucleus and bind to ARE, thereby promoting the expression levels of antioxidant genes such as NQO1, HO-1, and GSH-Px. Schogor *et al.*[33] found that curcumin can promote the dissociation of Nrf2 from Keap1 in porcine renal epithelial proximal tubule cells and enhance the expression of HO-1, thereby exerting antioxidant functions.

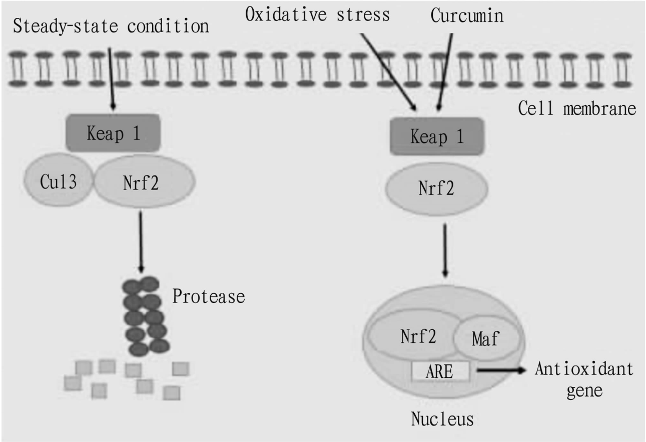

Fig. 1 Nrf2 signaling pathway and regulation of curcumin on Nrf2

2 Effects of curcumin on physiological functions of fish

2.1 Protective effect of curcumin on fish liver injury In fish culture process, liver tissue is highly susceptible to oxidative stress damage. Adding an appropriate amount of curcumin to fish feed has a certain protective effect against liver tissue injury. Yu Yunzhen *et al.*[23] investigated the protective effect of curcumin on damaged liver in grass carp. They first treated grass carp with carbon tetrachloride (CCl_4) to establish an acute liver injury model. After feeding the grass carp with diets containing curcumin, they found that supplementing the feed with 150 mg/kg of curcumin significantly inhibited the activities of glutamate-pyruvate transaminase (GPT) and glutamate-oxaloacetate transaminase (GOT) in serum. Therefore, curcumin exerts a protective effect against liver tissue damage in grass carp. Zhang Yuanyuan *et al.*[24] supplemented diets with 0, 60, 120, and 240 mg/kg curcumin and fed them to juvenile common carp. They found that 120 mg/kg curcumin repaired liver damage in juvenile carp, significantly promoted

Nrf2 gene expression levels in liver tissue, induced Nrf2 entry into liver cell nuclei, and enhanced the antioxidant stress capacity of carp hepatocytes. Besides, Zhang Yuanyuan *et al.* [28] supplemented Nile tilapia diets with 0, 15, 30, 60, 120, and 240 mg/kg curcumin and fed them to juvenile tilapia. Their results showed that dietary supplementation with 60 and 120 mg/kg curcumin improved growth performance and effectively inhibited acute liver injury and hepatic lipid peroxidation in fish. Curcumin also exhibits reparative effects against thioacetamide-induced hepatopancreas injury in grass carp[25].

2.2 Effects of curcumin on antioxidant capacity and immunity of fish Curcumin is a potent antioxidant. When added to fish feed, it can eliminate excess ROS in fish, significantly enhance the antioxidant capacity of the fish body, and protect its tissues and cells from oxidative damage. In addition, curcumin also has the effect of enhancing immunity, which can improve the disease resistance of fish. By regulating the activity of immune cells and the secretion of immune factors, curcumin can enhance the immune response capacity of aquatic animals and reduce the incidence of diseases. Yu Jun *et al.* [26] investigated the effects of adding curcumin at 0, 100, 150, and 300 mg/kg to fish feed for large yellow croakers. They found that curcumin could promote the growth of the fish and enhance their non-specific immune function. The supplementation of curcumin at a level of 300 mg/kg in the feed yielded the best results, significantly improving the disease resistance, non-specific immunity, and growth performance of the large yellow croakers. Yang Yusheng *et al.* [27] studied the effects of dietary supplementation with curcumin, chitosan, vitamin C, and vitamin B_2 on the growth, digestion, and antioxidant capacity of yellow catfish. The results showed that a diet supplemented with 150 mg/kg curcumin, 4 500 mg/kg chitosan, 709 mg/kg vitamin C, and 240 mg/kg vitamin B_2 had relatively good effects. Zhu Huaining *et al.* [28] supplemented the diets of juvenile American eels with curcumin at levels of 100, 200, 300, and 400 mg/kg to study its impact on liver health. They found that curcumin could reduce the levels of liver inflammatory factors and decrease the activities of serum GPT and GOT. Chen Yanxuan *et al.* [29] added curcumin to the diet of yellow catfish containing oxidized fish oil and found that curcumin could increase serum CAT and lysozyme activities, as well as enhance the antioxidant capacity and immunity of the fish.

Dong Kaiyue *et al.* [29] supplemented the diets of juvenile hybrid sturgeon with curcumin at levels of 0, 0.025, 0.05, 0.1, and 0.2 g/kg. Their findings revealed that dietary curcumin exerted antioxidant, hepatoprotective, lipid-lowering, and digestion-promoting effects on the juvenile hybrid sturgeon. The optimal supplementation level of curcumin in the diet was determined to be 0.025 – 0.05 g/kg. When turbot were fed diets supplemented with curcumin at levels of 0, 0.02%, 0.04%, and 0.06%, the body fat content of the fish decreased significantly, while liver and muscle fat content showed a significant increasing trend. Additionally, curcumin significantly increased serum CAT activity and reduced serum malondialdehyde (MDA) content, exerting a significant effect on the serum antioxidant capacity of juvenile turbot[30]. Zhang Tengxian *et al.* [31] supplemented yellow catfish feed with curcumin at 0, 50, 100, 200, 400, and 800 mg/kg and found that curcumin improved the survival rate and specific growth rate of yellow catfish. Curcumin at 200 mg/kg significantly enhanced the antioxidant capacity, growth performance, and digestive ability of the fish. Supplementation of 100 mg/kg curcumin in the diet of largemouth bass significantly induced the expression levels of Keap1 in the gills and intestine, inhibited the expression of Nrf2, and significantly induced the gene expression levels of Nrf2, interleukin-10 (IL-10), transforming growth factor-β1 (TGF-β1), and the apoptosis regulator Bcl-2 in the liver, indicating that curcumin has favorable anti-inflammatory and anti-apoptotic effects in largemouth bass[32].

2.3 Effects of curcumin on growth performance of fish Appropriate levels of dietary curcumin can promote fish growth. Studies have shown that adding an appropriate amount of curcumin to fish feed can significantly improve the growth rate, weight gain rate, and feed conversion rate of fish. This is mainly attributed to curcumin's ability to stimulate fish appetite, increase feed intake, and promote the digestion and absorption of nutrients. Wang Jinbo and Wu Tianxing[24] supplemented large yellow croaker feed with 0.02%, 0.04%, and 0.06% curcumin and found that it increased the body weight gain rate and significantly reduced the feed conversion ratio. The optimal growth effect was achieved with approximately 0.04% curcumin supplementation in the diet of large yellow croaker. Zhang Yuanyuan *et al.* [34] found that dietary supplementation with 60 and 120 mg/kg curcumin increased the weight gain rate of tilapia and improved their growth performance. Yu Jun *et al.* [26] added curcumin at levels of 0, 100, 150, and 300 mg/kg to fish feed for large yellow croaker and found that the 300 mg/kg supplementation level had the best growth-promoting effect. Additionally, Ming Jianhua *et al.* [35] supplemented grass carp feed with 400 and 600 mg/kg curcumin and found that it improved the growth performance and anti-oxidative stress capacity of grass carp, reduced the feed conversion ratio, provided protection against acute oxidative stress injury, and activated the expression levels of genes related to the Nrf2/ARE signaling pathway.

2.4 Effects of curcumin on improving color and quality of fish Curcumin, a natural yellow pigment, exhibits excellent coloring properties. Its inclusion in fish feed effectively enhances the body coloration of fish, thereby significantly increasing the market value of aquatic products. Furthermore, curcumin positively influences the flesh quality and taste of fish, improving overall meat quality when incorporated into the diet. Research on juvenile American eels demonstrated that dietary curcumin supplementation at 200 mg/kg (among levels of 100, 200, 300, and 400 mg/kg) produced the most favorable results in terms of body color enhancement[36]. In a study on large yellow croaker, Wang and Wu[34] reported that increasing dietary curcumin levels (0.02%, 0.04%, and 0.06%) led to significant improvements in both body color and sensory quality. Hu *et al.* [37] investigated largemouth bass and found that supplementing feed with 50 mg/kg curcumin reduced muscle monounsaturated fatty acid content while increasing polyunsaturated fatty acid levels, thereby enhancing muscle quality. Additionally, Chen *et al.* [38] demonstrated that curcumin supplementation in yellow catfish diets containing oxidized fish oil effectively counteracted body color degradation induced by the oxidized oil, while also modifying muscle texture by reducing cohesiveness, springiness, and chewiness, and increasing hardness.

3 Conclusions and prospects

Curcumin is now extensively incorporated into the feed of various aquatic animals, with remarkable outcomes observed when added to the diets of some fish species. Nevertheless, challenges like its limited water solubility and low bioavailability call for further exploration and the creation of novel formulations to improve its stability and performance in feed. With growing consumer focus on food safety, minimizing antibiotic application in aquaculture has emerged as an industry-wide goal. As a natural antimicrobial compound, curcumin can partially replace antibiotics in aquatic animal feed. By suppressing the proliferation of pathogenic bacteria, curcumin helps lower disease incidence in aquatic species and curbs antibiotic reliance, thus safeguarding the safety and quality of aquatic products. As research on curcumin deepens and technology evolves, its potential for use in aquatic animal feed is set to expand further.

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