Chapter 60

Potential of Antioxidants-unleashing Natural Defense against Oxidative Stress in Fish

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ABSTRACT

Oxidative stress significantly impacts fish production, influencing growth, health, and meat quality. Antioxidants are vital in maintaining cellular homeostasis and protecting fish from oxidative stress damages. This literature highlights the potential of antioxidants in enhancing natural defense mechanisms against oxidative stress in fish. It examines various sources of oxidative stress, including environmental, nutritional, and physiological factors, and underscores the importance of endogenous antioxidant defense systems, consisting of enzymatic and non-enzymatic antioxidants. The potential of exogenous antioxidants from plants, algae, and microorganisms in fish nutrition is explored, along with their effects on growth, immune response, stress resistance, and flesh quality. Challenges and future perspectives related to antioxidant supplementation in fish nutrition are discussed, emphasizing the need for optimizing supplementation strategies, understanding interactions with other dietary components, monitoring potential adverse effects, and developing novel delivery systems. Future research directions include elucidating the mechanisms of action of different antioxidants, exploring synergistic or additive effects, and advancing sustainable strategies for promoting fish health and production through strategic antioxidant use in fish diets. Hence, this chapter provides valuable insights for researchers, nutritionists, and aquaculture practitioners aiming to improve fish welfare and optimize production through effective antioxidant use in fish nutrition.

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INTRODUCTION

Oxidative Stress in Fish

Oxidative stress is a phenomenon that occurs when the balance between the production of reactive oxygen species (ROS) and the antioxidant defense mechanisms is disrupted, leading to potential cellular damage (Sachdev et al., 2021). In fish, oxidative stress can be induced by various environmental, nutritional, and physiological factors, which can negatively impact their health, growth, and overall performance (Gopalraaj et al., 2024). Fish are particularly susceptible to oxidative stress due to their aquatic habitat, which exposes them to numerous stressors such as temperature fluctuations, hypoxia, and pollution (Abdikalikova et al., 2024).

ROS are highly reactive molecules that can cause oxidative damage to cellular components, including lipids, proteins, and DNA (Jomova et al., 2024). In fish, ROS are generated as by-products of normal metabolic processes, such as cellular respiration and immune responses (Hong et al., 2024). However, when ROS production exceeds the capacity of the antioxidant defense systems, oxidative stress ensues (Yang et al., 2024). This imbalance can lead to various pathological conditions, such as immunosuppression, growth retardation, and increased susceptibility to diseases (Cai et al., 2024). Studies have shown that oxidative stress in fish can be induced by a wide range of environmental factors, including temperature extremes (Islam et al., 2022), hypoxia and exposure to pollutants such as heavy metals (Turan et al., 2020) and pesticides (Yang et al., 2020).

Nutritional factors can also contribute to oxidative stress in fish. Diets deficient in essential nutrients, such as vitamins C and E, can impair the antioxidant defense systems and increase the susceptibility to oxidative damage (Xiao et al., 2024).

On the other hand, diets containing high levels of polyunsaturated fatty acids (PUFAs) can increase the risk of lipid peroxidation, as PUFAs are highly susceptible to oxidation (Islam et al., 2023). Sea bream (*Sparus aurata*) fed a diet high in PUFAs exhibited increased levels of lipid peroxidation and reduced activity of antioxidant enzymes compared to fish fed a diet with lower PUFA content (Bouraoui et al., 2023).

Physiological factors, such as growth, reproduction, and immune responses, can also contribute to oxidative stress in fish. During periods of rapid growth or reproduction, the metabolic demands increase, leading to higher ROS production. Additionally, the activation of the immune system in response to pathogens or stressors can generate ROS as part of the defense mechanism. However, if the ROS production is not adequately regulated, it can result in oxidative damage to the host tissues (Abdel-Tawwab et al., 2019).

Antioxidants are substances that can prevent or delay oxidative damage caused by ROS (Gulcin 2020). In fish, antioxidants play a crucial role in maintaining the balance between ROS production and elimination, thereby protecting the cells and tissues from oxidative stress Li et al., 2023). Antioxidants can be classified into two main categories i.e. enzymatic and non-enzymatic antioxidants (Naz et al., 2023).

The antioxidant defense systems in fish are influenced by various factors, such as species, age, tissue type, and environmental conditions. For example, marine fish generally have higher antioxidant enzyme activities compared to freshwater fish, possibly due to the higher oxygen solubility in seawater. Additionally, the antioxidant defense systems can be modulated by dietary factors, such as the intake of antioxidant nutrients (Tocher, 2003). Dietary supplementation with vitamins C and E, carotenoids, and other antioxidant compounds can enhance the antioxidant status and protect fish against oxidative stress (Gopalraaj et al., 2024).

Sources of Oxidative Stress in Fish

Oxidative stress in fish can be induced by various factors, both internal and external. These factors can disrupt the balance between ROS production and antioxidant defenses, leading to potential cellular damage and negative impacts on fish health and performance (Birnie-Gauvin et al., 2017).

Environmental Factors

Environmental factors play a significant role in inducing oxidative stress in fish. Fish are exposed to a wide range of environmental stressors in their aquatic habitats, which can influence their oxidative status. One of the major environmental factors that can cause oxidative stress in fish is temperature. Fish are ectothermic animals, meaning their body temperature is regulated by the surrounding environment. When exposed to temperature extremes, either too high or too low, fish can experience increased ROS production and oxidative damage (Menon et al., 2023).

Hypoxia, or low dissolved oxygen levels, is another environmental stressor that can induce oxidative stress in fish. During hypoxic conditions, fish may experience increased ROS production due to the disruption of mitochondrial electron transport chain and the activation of xanthine oxidase. This can lead to oxidative damage in various tissues, such as the brain, liver, and gills (Nitz et al., 2023). Exposure to hypoxia resulted in increased lipid peroxidation and altered antioxidant enzyme activities in the brain of common carp (Cyprinus carpio) (Jia et al., 2021).

Exposure to pollutants, such as heavy metals and pesticides, can also induce oxidative stress in fish. These contaminants can enter the aquatic environment through various sources, including industrial effluents, agricultural runoff, and sewage discharge. Heavy metals, such as cadmium, copper, and mercury, can accumulate in fish tissues and generate ROS through Fenton reactions or by depleting antioxidant defenses. Pesticides, such as organochlorines and organophosphates, can also induce oxidative stress in fish by disrupting the antioxidant system and increasing ROS production (Rani et al., 2022).

Nutritional Factors

Nutritional factors can significantly influence the oxidative status of fish. Imbalanced or deficient diets can lead to oxidative stress by affecting the antioxidant defense system and increasing the susceptibility to oxidative damage (Yu et al., 2022). One of the essential nutrients that play a crucial role in maintaining the oxidative balance in fish is vitamin C (ascorbic acid). Vitamin C is a potent antioxidant that can directly scavenge ROS and regenerate other antioxidants, such as vitamin E (Hamre, 2011). Fish cannot synthesize vitamin C endogenously and must obtain it from their diet. Studies have shown that dietary vitamin C deficiency can lead to increased oxidative stress and reduced growth and survival in various fish species (Kükürt and Gelen, 2024).

Vitamin E (tocopherols and tocotrienols) is another important nutrient that helps protect fish against oxidative stress. As a lipid-soluble antioxidant, vitamin E plays a crucial role in maintaining the integrity of cell membranes by preventing lipid peroxidation. Dietary vitamin E deficiency has been associated with increased oxidative damage, reduced growth, and impaired immune function in fish (Sivaramakrishnan et al., 2024).

The type and quality of dietary lipids can also influence the oxidative status of fish. Fish require essential fatty acids, particularly long-chain polyunsaturated fatty acids (LC-PUFAs), for optimal growth and health. However, LC-PUFAs are highly susceptible to oxidation due to their multiple double bonds. Dietary oxidized lipids can increase the oxidative load in fish and lead to oxidative stress, affecting growth, immune function, and flesh quality (Yan et al., 2024. Rainbow trout (*Oncorhynchus mykiss*) fed a diet containing oxidized soybean oil exhibited reduced growth, altered antioxidant enzyme activities, and increased lipid peroxidation in the liver and intestine Jiang et al. (2024).

Physiological Factors

Physiological factors, such as growth, reproduction, and immune responses, can also contribute to oxidative stress in fish. During periods of rapid growth or reproduction, fish may experience increased metabolic rates and oxygen consumption, which can lead to higher ROS production. For example, the activity of antioxidant enzymes (SOD and CAT) was found to increase in the liver of rainbow trout during the period of rapid growth, suggesting an adaptive response to increased oxidative stress (Huang et al., 2021).

Reproductive processes, such as gonadal maturation and spawning, can also influence the oxidative status of fish. During these periods, fish may experience increased oxidative stress due to the high energy demands and the production of ROS associated with steroidogenesis and gamete development (Nartea et al., 2023). The fish immune system also plays a role in inducing oxidative stress. During an immune response to pathogens or inflammatory stimuli, fish phagocytic cells (e.g., neutrophils and macrophages) produce ROS as part of their bactericidal activity. While this is an essential mechanism for defending against pathogens, excessive or prolonged ROS production can lead to oxidative damage in the surrounding tissues. The expression of antioxidant genes (SOD, CAT, and GPx) was found to increase in the head kidney of gilthead sea bream (*Sparus aurata*) following a challenge with the pathogen *Vibrio anguillarum*, suggesting a role for the antioxidant system in modulating the immune response (Montero et al., 2024).

Endogenous Antioxidant Defense Systems in Fish

Fish, like other organisms, possess endogenous antioxidant defense systems that help protect them from the deleterious effects of oxidative stress. These defense systems comprise a complex network of antioxidant enzymes and non-enzymatic antioxidants that work together to maintain cellular redox homeostasis and prevent oxidative damage to cellular components, such as lipids, proteins, and DNA (Song et al., 2023).

Enzymatic Antioxidants

Enzymatic antioxidants are proteins that catalyze reactions to neutralize reactive oxygen species (ROS) and convert them into less harmful molecules. The main enzymatic antioxidants in fish include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) (Subaramaniyam et al., 2023).

Superoxide dismutase (SOD) is the first line of defense against ROS, specifically superoxide anion (O_2). SOD catalyzes the dismutation of O_2 into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). In fish, there are three types of SOD: cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD (EC-SOD). The activity of SOD has been widely used as a biomarker of oxidative stress in fish exposed to various environmental stressors, such as temperature changes, hypoxia, and pollutants (Ahmed et al., 2023).

Catalase (CAT) is an enzyme that catalyzes the decomposition of H2O2 into water (H2O) and O2. CAT is mainly localized in the peroxisomes and plays a crucial role in protecting cells from the toxic effects of H_2O_2. In fish, CAT activity has been found to vary depending on the species, tissue type, and environmental conditions. For example, the activity of CAT in the liver of the Antarctic fish *Notothenia coriiceps* was higher than that of temperate fish species, suggesting an adaptive response to the extreme cold environment (Lattuca et al., 2023).

Glutathione peroxidase (GPx) is another important enzyme that catalyzes the reduction of H_2O_2 and organic hydroperoxides, using reduced glutathione (GSH) as a cofactor. GPx plays a crucial role in protecting cell membranes from lipid peroxidation. In fish, several isoforms of GPx have been identified, including cytosolic GPx, mitochondrial GPx, and phospholipid hydroperoxide GPx (PHGPx). The activity of GPx has been shown to be modulated by various factors, such as diet, temperature, and exposure to pollutants (Hu et al., 2024). Glutathione reductase (GR) is an enzyme that catalyzes the reduction of oxidized glutathione (GSSG) back to its reduced form (GSH), using NADPH as a cofactor. GR plays a crucial role in maintaining the cellular GSH/GSSG ratio, which is an important indicator of the cellular redox status. In fish, GR activity has been found to vary depending on the species, tissue type, and environmental conditions (Canosa and Bertucci 2023).

Non-enzymatic Antioxidants

Non-enzymatic antioxidants are low molecular weight compounds that can scavenge ROS or prevent their formation. In fish, important non-enzymatic antioxidants include glutathione (GSH), vitamin C (ascorbic acid), vitamin E (tocopherols and tocotrienols), carotenoids, and uric acid (Andrés et al., 2024).

Glutathione (GSH) is a tripeptide consisting of glutamate, cysteine, and glycine. GSH is the most abundant low molecular weight thiol in cells and plays a crucial role in maintaining cellular redox homeostasis. GSH can directly scavenge ROS, such as hydroxyl radicals (OH) and singlet oxygen ($1O_2$), and serve as a cofactor for GPx in the reduction of H_2O_2 and organic hydroperoxides. In fish, GSH levels have been shown to vary depending on the species, tissue type, and environmental conditions. Exposure to heavy metals, such as cadmium and copper, resulted in a significant decrease in GSH levels in the liver and gills of the freshwater fish *Oreochromis niloticus* (Kocalar et al., 2023).

Vitamin C (ascorbic acid) is a water-soluble antioxidant that can directly scavenge ROS, such as O_2, H_2O_2, and OH, and regenerate other antioxidants, such as vitamin E (Mahmood et al., 2023). Fish cannot synthesize vitamin C endogenously and must obtain it from their diet. Dietary vitamin C supplementation has been shown to enhance the antioxidant defense system and protect fish against oxidative stress induced by various environmental stressors, such as high temperature, hypoxia, and pollutants (El-Sayed and Izquierdo 2022).

Vitamin E (tocopherols and tocotrienols) is a lipid-soluble antioxidant that plays a crucial role in protecting cell membranes from lipid peroxidation. Vitamin E can scavenge lipid peroxyl radicals and break the chain reaction of lipid peroxidation. In fish, dietary vitamin E supplementation has been shown to enhance the antioxidant defense system and improve the resistance to oxidative stress induced by various factors, such as high temperature, hypoxia, and pollutants (El-Sayed and Izquierdo 2022).

Carotenoids, such as astaxanthin and lycopene, are lipid-soluble pigments that possess antioxidant properties. Carotenoids can scavenge ROS, particularly singlet oxygen ($1O_2$), and protect cell membranes from lipid peroxidation. In fish, carotenoids are obtained from the diet and are known to contribute to the pigmentation of the skin and flesh. Dietary carotenoid supplementation has been shown to enhance the antioxidant defense system and improve the resistance to oxidative stress in various fish species (Taalab et al., 2022; Lim et al., 2023).

Uric acid is a water-soluble antioxidant that can scavenge ROS, such as OH and $1O_2$, and chelate transition metal ions, such as iron and copper, which can catalyze the formation of ROS. In fish, uric acid is produced as an end product of purine metabolism and has been shown to contribute to the antioxidant defense system (Nwizugbo et al., 2023).

Exogenous Antioxidants and their Potential in Fish

In addition to the endogenous antioxidant defense systems, fish can also benefit from exogenous antioxidants provided through their diet. Exogenous antioxidants are compounds that can be obtained from external sources, such as food or dietary supplements, and have the potential to enhance the antioxidant defense system and protect against oxidative stress (Monier 2020).

Natural Antioxidants

Natural antioxidants are compounds that are derived from natural sources, such as plants, algae, and microorganisms. These antioxidants have gained increasing attention in recent years due to their potential health benefits and consumer preference for natural products. In fish nutrition, several natural antioxidants have been investigated for their potential to enhance the antioxidant defense system and improve fish health and performance (Ahmadifar et al., 2021).

Plant-derived Antioxidants

Plants are rich sources of natural antioxidants, including phenolic compounds, flavonoids, carotenoids, and tocopherols. These antioxidants have been shown to possess potent free radical scavenging and metal chelating activities, making them promising candidates for use in fish nutrition. Phenolic compounds, such as phenolic acids and flavonoids, are among the most widely studied plant-derived antioxidants. These compounds have been found to exhibit strong antioxidant activities and have been shown to protect fish against oxidative stress induced by various factors, such as high temperature, hypoxia, and pollutants. Dietary supplementation with thymol, a phenolic compound found in thyme, significantly enhanced the antioxidant defense system and improved the growth performance of Nile tilapia (*Oreochromis niloticus*) under high temperature stress (Shourbela et al., 2021).

Carotenoids, such as astaxanthin and lycopene, are another class of plant-derived antioxidants that have been investigated for their potential use in fish nutrition. These compounds have been shown to possess strong singlet oxygen quenching and free radical scavenging activities. Dietary supplementation with carotenoids has been found to enhance the antioxidant defense system and improve the resistance to oxidative stress in various fish species (Meléndez-Martínez et al., 2022).

Algae-derived Antioxidants

Algae are another promising source of natural antioxidants for use in fish nutrition. Algae contain a wide range of antioxidant compounds, including carotenoids, phycobiliproteins, polyphenols, and sulfated polysaccharides (López-Pedrouso et al., 2020). These antioxidants have been shown to possess potent free radical scavenging and metal chelating activities and have been investigated for their potential to enhance the antioxidant defense system in fish. Among the algae-derived antioxidants, carotenoids, particularly astaxanthin, have received the most attention in fish nutrition.

Phycobiliproteins, such as phycocyanin and phycoerythrin, are water-soluble pigments that are found in cyanobacteria and red algae. These compounds have been shown to possess strong antioxidant and anti-inflammatory activities. Dietary supplementation with phycobiliproteins has been found to enhance the antioxidant defense system and improve the immune response in various fish species, such as rainbow trout and tilapia (Amer 2016; Vazirzadeh et al., 2020)

Microbial-derived Antioxidants

Microorganisms, such as bacteria and fungi, are also potential sources of natural antioxidants for use in fish nutrition. These antioxidants include carotenoids, enzymes, and exopolysaccharides. Microbial-derived antioxidants have been investigated for their potential to enhance the antioxidant defense system and improve fish health and performance.

Astaxanthin is not only produced by microalgae but also by several bacteria and fungi species, such as *Paracoccus carotinifaciens* and *Xanthophyllomyces dendrorhous* (formerly Phaffia rhodozyma) (Barredo et al., 2017). Microbial enzymes, such as superoxide dismutase (SOD) and catalase (CAT), have also been investigated for their potential use as antioxidant supplements in fish nutrition. (Gobi et al., 2018).

Synthetic Antioxidants

Synthetic antioxidants are chemically synthesized compounds that have been widely used in the food and feed industry to prevent lipid oxidation and extend the shelf life of products. The most commonly used synthetic antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ethoxyquin (EQ), and tert-butylhydroquinone (TBHQ). These antioxidants have been shown to possess strong free radical scavenging activities and have been used in fish feeds to prevent oxidative deterioration of lipids (Hossein et al., 2022).

However, the use of synthetic antioxidants in fish nutrition has been a subject of controversy due to concerns about their potential toxicity and consumer preference for natural products (Biller and Takahashi, 2018). Some studies have suggested that prolonged exposure to high levels of synthetic antioxidants may have adverse effects on fish health and performance. For example, a study by Nunes et al. (2015) demonstrated that high dietary levels of EQ (>150 mg/kg) can lead to reduced growth and feed efficiency in Atlantic salmon. There has been a growing interest in replacing synthetic antioxidants with natural alternatives in fish feeds. Natural antioxidants derived from plants, algae, and microorganisms have been shown to be effective in preventing lipid oxidation and enhancing the antioxidant defense system in fish, without the potential negative effects associated with synthetic antioxidants. However, further research is needed to optimize the use of natural antioxidants in fish nutrition and to evaluate their long-term effects on fish health and performance (Khan et al., 2017).

Antioxidant Supplementation in Fish Diets

The supplementation of antioxidants in fish diets has gained increasing attention in recent years due to their potential to enhance growth, immune response, stress resistance, and flesh quality. Antioxidants play a crucial role in maintaining cellular homeostasis and protecting against oxidative stress, which can have detrimental effects on fish health and performance (Shastak and Pelletier 2023).

Effects on Growth and Performance

Dietary supplementation with antioxidants has been shown to improve growth and performance in various fish species. The inclusion of antioxidants in fish diets can help alleviate oxidative stress, which can negatively impact feed intake, nutrient utilization, and growth. For example, astaxanthin significantly improved the growth performance and feed efficiency of juvenile large yellow croaker (*Larimichthys crocea*) (Zhu et al., 2024). The positive effects of vitamin E on growth performance have been attributed to its role in maintaining the integrity of cell membranes and protecting against oxidative damage (Rahman et al., 2023).

Effects on Immune Response

The fish immune system is highly susceptible to oxidative stress, and dietary antioxidants have been shown to play a crucial role in modulating immune responses. Antioxidants can help maintain the balance between pro-oxidants and antioxidants, which is essential for optimal immune function. Vitamin C has been shown to increase the activity of lysozyme, complement, and respiratory burst, which are important components of the fish innate immune system (Zhu et al., 2024).

Effects on Stress Resistance

Fish are exposed to various stressors in their environment, such as temperature fluctuations, hypoxia, and handling, which can lead to oxidative stress and compromised health. Dietary vitamin E supplementation has been found to improve the resistance of fish to oxidative stress induced by various factors, such as high temperature, hypoxia, and pollutants. Vitamin E supplementation significantly reduced the level of lipid peroxidation in the liver of grass carp exposed to high water temperature (Yao et al., 2024). Similarly, dietary vitamin C supplementation has been shown to enhance the resistance of common carp to oxidative stress induced by handling and transport (Labh 2024).

Effects on Flesh Quality

Flesh quality is an important aspect of fish production, as it directly affects consumer acceptance and market value. Oxidative stress can lead to the deterioration of flesh quality by promoting lipid and protein oxidation, which can result in off-flavors, discoloration, and texture changes. Dietary antioxidants have been shown to improve the flesh quality of fish by reducing oxidative damage and maintaining the stability of lipids and proteins. Dietary vitamin E supplementation has been found to improve the flesh quality of European sea bass (*Dicentrarchus labrax*) (Moroni et al., 2024). Vitamin E has been shown to reduce lipid oxidation and maintain the color and texture of fish fillets during storage (Nahavandi et al., 2024).

Challenges and Future Perspectives

While the supplementation of antioxidants in fish diets has shown promising results in enhancing growth, immune response, stress resistance, and flesh quality, there are still several challenges and knowledge gaps that need to be addressed. One of the main challenges in antioxidant supplementation is determining the optimal dietary levels and combinations of antioxidants for different fish species and life stages. The antioxidant requirements of fish can vary depending on factors such as species, age, size, physiological status, and environmental conditions. Therefore, it is essential to establish species-specific and life stage-specific recommendations for antioxidant supplementation. Moreover, the bioavailability and efficacy of different antioxidant sources can vary significantly. For instance, the bioavailability of astaxanthin from natural sources, such as microalgae and crustacean meals, has been shown to be higher than that of synthetic astaxanthin in Atlantic salmon and red sea bream. Therefore, future research should focus on identifying and characterizing novel antioxidant sources with high bioavailability and potency (Elbahnaswy and Elshopakey 2024).

Another challenge in antioxidant supplementation is understanding the interactions between antioxidants and other dietary components. Antioxidants can interact with other nutrients, such as lipids, proteins, and minerals, which can affect their bioavailability and functionality. Moreover, the presence of pro-oxidants, such as transition metals (e.g., iron and copper), in the diet can counteract the beneficial effects of antioxidants by promoting oxidative stress. Therefore, future research should aim to elucidate the complex interactions between antioxidants and other dietary components and develop strategies to optimize the antioxidant-nutrient balance in fish feeds (Tamta et al., 2024).

Potential Adverse Effects of Excessive Antioxidant Supplementation

While antioxidants are generally considered safe and beneficial, excessive supplementation can lead to potential adverse effects. High dietary levels of certain antioxidants, such as vitamin E, have been shown to impair growth performance and feed utilization in some fish species. This may be due to the pro-oxidant effects of antioxidants at high concentrations or their interference with the absorption and metabolism of other nutrients. Moreover, excessive antioxidant supplementation may also suppress the endogenous antioxidant defense system and increase the susceptibility of fish to oxidative stress. Therefore, it is crucial to establish safe upper limits for antioxidant supplementation in fish diets and monitor the potential adverse effects of long-term supplementation (Gopalraaj et al., 2024).

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