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ANTIOXIDANT ADDITIVES IN FISH FEEDS

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Abstract: Aquaculture is a rapidly developing sector in recent years. For humans, one of the most important sources of protein is fish and other products from aquaculture. Antioxidants are used to prevent oxidation problems in the feeds used in the production of these products. The task of antioxidants is to prevent rancidity of fats and to keep feeds stable. Fish fed with oxidized feeds experience many negative effects such as growth retardation, low feed utilization, weak immune system and reduced resistance to diseases. As a result, it can cause great economic losses along with fish losses. Synthetic antioxidants have been used successfully for many years. However, in recent years, some restrictions and regulations have been introduced the use of synthetic antioxidants. Therefore, natural antioxidants have begun to replace synthetic antioxidants. The sources of natural antioxidants are quite abundant, such as fruits, vegetables, plant extracts, marine macro and microalgae. In recent years, research on these natural antioxidants and their use in fish feeds have been increasing.

Keywords: BHA, BHT, Etoxyquin, Natural antioxidants

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1. Introduction

In aquaculture, unbalanced and oxidized feeds and stress conditions make fish more vulnerable to diseases and cause economic losses due to fish deaths (Amer et al., 2018). It is important to use balanced feed formulations, vaccines and immunostimulants to reduce disease risks in cultured fish, thus increasing the resistance levels of fish against infections (Amer et al., 2018; Amer et al., 2019; Al-Khalaifah et al., 2020; El-Araby et al., 2020).

The unconscious use of broad-spectrum chemicals that have toxic or lethal effects on disease-causing bacteria, protozoa, viruses and parasites without harming the host has caused the resistance of microorganisms to these drugs and the increase of pathogens. In other words, it causes the formation of drug resistance (Ai et al., 2011). In this case, the spread of drug-resistant pathogens has caused many other problems, such as environmental hazards and food safety issues.

Prevention of diseases is more important than treatment. Especially when it comes to diseases caused by feed. Decomposed, poorly stored, nutritionally deficient feed can cause disease. Feeds used in aquaculture contain partially or high amounts of fish oil or other vegetable oils. These added oils are based on marine ingredients containing high levels of polyunsaturated ω-3 fatty acids and are therefore susceptible to lipid oxidation. Oxidized feeds adversely affect the resistance of fish against diseases, at the same time they damage both growth and development and fillet quality. (Sutton et al., 2006; Grigorakis et al., 2010). It has been shown that the use of oxidized oils in the feeds increases the plasma glucose, cortisol and osmolarity levels of the fish, therefore the stress factor develops negatively (Van Anholt et al., 2004; Alves Martins et al., 2007).

During the production, transportation and storage of feed, oxidation can be a major process that can reduce its quality. Oxidation can take place through various mechanisms, including autoxidation, photosensitized oxidation, thermal oxidation, and enzymatic oxidation.

Autoxidation occurs when feed comes into contact with atmospheric oxygen, resulting in the formation of radicals. This reaction can lead to the oxidation of feed, which can cause a decrease in its nutritional and physical quality (Cho and Min, 2009). In order to prevent these problems in fish caused by feed, additives with antioxidant properties are added to fish feeds. These additives used both prevent the oxidation of the feed and ultimately help protect the health of the fish. In this article, general information about antioxidants used in fish feeds and information about their effects are given.

2. Antioxidants

Antioxidants are substances that act as shields against the negative reactions of oxygen (oxidation) of complex structures such as proteins, lipids, carbohydrates and DNA in a cell. The organism is constantly under the influence of both internal (digestive, respiratory, disease, injury, etc.) and external (environmental factors) factors.

As a result, oxidant molecules formed during and after these effects damage cells and tissues. In living organisms, there is a system that constantly neutralizes reactive oxygen species (ROS) and other pro-oxidants through low molecular weight free radical scavengers and antioxidant enzymes. Both intracellular and extracellular enzyme and non-enzyme defense mechanisms against reactive oxygen species or oxidant molecules are called antioxidant defense system (Mates et al. 1999; Dündar et al., 1999; Dündar et al., 2000; Ritola et al., 2002; Mclean et al., 2005; Pham-Huy et al., 2008).

If antioxidant defense is mediated by catalase (CAT), superoxide dismutase (SOD), Glutathione peroxidase (GPx) and Glutathione reductase (GR) enzymes, it is called enzymatic antioxidant defense. If the defense is with substances such as tocopherol (Vitamin E), ascorbic acid (Vitamin C), retinol (Vitamin A), it is expressed as a non-enzymatic antioxidant defense system (Valko et al. 2007). There are antioxidants in many different foods that we obtain from nature. As for the classification of antioxidants, many different classifications can be seen in the literature. These; such as where it is obtained from, its mode of action, activities, biochemical properties (Kebede and Admassu, 2019).

It is possible to examine antioxidants in two groups as natural antioxidants and synthetic antioxidants. In general, among natural antioxidants, enzymes, macromolecules and micromolecules can be given as examples (Hilmi, 1994; Sen and Chakraborty, 2011). Some of the plant organisms found in nature (especially vegetables and fruits) are good sources of natural antioxidants. (Grozea, 2012; Akbarirad et al., 2016; Kebede and Admassu, 2019). These foods are very rich of antioxidant compounds such as Vit E, Vit A, Vit C, β-carotene and etc. (Sies et al., 1992; Anbudhasan et al., 2014). The most important antioxidant compounds found in plant extracts are polyphenols (flavonoids) and phenolic acids and carotenoids. (Balasundram et al. 2006; Göktürk et al. 2007; Sicuro et al., 2010).

Some important natural antioxidants and their sources presented in Table 1. (Balasundram et al., 2006; Grozea, 2012; Akbarirad et al., 2016; Kebede and Admassu, 2019). As regards to synthetic antioxidants are chemically synthesized additives to food products as preservatives to help prevent oxidation (Kebede and Admassu, 2019). Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), etoxyquin (EQ) and citric acid are the most well-known (Sicuro et al., 2010; Blaszczyk et al., 2013).

In addition, these synthetic antioxidants have been used for many years to increase stability in foods and animal feeds (Blaszczyk et al., 2013). However, there is conflicting information about their use. In recent years, updates have been made on the usage areas and doses of these additives. Reported due to growing evidence of its adverse effects on aquatic life and humans, the EU commission has suspended the authorization of ethoxyquin as a feed additive for all animal species and categories. According to Gunathilake et al. (2022) due to growing evidence of its adverse effects on aquatic life and humans, the EU commission has suspended the authorization of ethoxyquin as a feed additive for all animal species and categories. On the other hands, new informations have been presented about using of ethoxyquin.

Table 1. Sources of important natural antioxidants

Compounds	Natural Source
Carotenoids	Dark leafy vegetable, carrots, sweet potatoes, yams, tomatoes, apricots, citrus fruits, kale, papaya
Catechins	Green tea, berries, certain oilseeds
Flavonoids (Polyphenols)	Oilseeds, lettuce, berries, eggplants, peppers, citrus fruits, cruciferous vegetables, onions, black tea
Lycopene	Tomatoes, papaya, watermelon, guava
Phenolic acids	Oilseeds and certain oils, cereals, grains
Vitamin C (ascorbic acid)	Fruits and vegetables, berries, citrus fruits, green peppers, potatoes
Vitamin E (tocopherols)	Oilseed, palm oil, nuts, eggs, dairy products, whole grains, vegetables, cereals, margarine and etc.
Extracts	Extract from green tea, rosemary, sage, clove, oregano, thyme, oat, rice bran and etc.

According to the regulation made by the European Commission on 5 August 2022, it is stated as follows: The existing authorisation of the additive ethoxyquin was suspended by Commission Implementing Regulation (EU) 2017/962.

In accordance with Implementing Regulation (EU) 2017/962, the suspension measure is to be reviewed by 31 December 2022 and in any event after the adoption by the Authority of a non-favourable opinion on the safety and efficacy of the additive ethoxyquin. (EU, 2022).

In the regulation made by the European Commission on October 5, 2022, it was stated that BHA can be used in other animal feeds except cats at the rate of 150 mg/kg. This rate should be; 150 mg of active substance / kg of complete feeding stuff with a moisture content of 12% moisture (EU, 2020). Also in the same regulation was stated that, BHA can be used in combination with butylated hydroxytoluene (BHT) up to 150 mg of the mixture/kg of complete feed (EU, 2020).

3. Antioxidants in Aquaculture

Nowadays, antioxidants prevent spoilage in foods and are added to every product consumed to keep them intact for a longer period of time. Among the food compounds, lipids have the highest risk of being oxidized. In general, oxidation of lipids occurs during improper storage, processing, heat treatment of raw materials, and packaging and storage of processed materials. During the oxidation formation, many compounds such as peroxides, hydrocarbons, aldehydes, ketones, alcohols and acids are formed. These compounds lose their bitterness and sensory properties in foods, these changes reduce food quality and shorten shelf life (Turan et al., 2012).

Fish tissues are more susceptible to lipid peroxidation than other compounds due to the high content of polyunsaturated fatty acids. Among living things, animals have formed a major defense mechanism against in vivo peroxidation caused by antioxidant enzymes, endogenous antioxidants and nutritional antioxidants (Hamre et al., 2004). In order not to deteriorate the food quality and shorten the shelf life of seafood, an effective antioxidant is needed, especially to prevent deterioration of lipids in fish tissues.

Lipids in feeds can be subject to oxidation during production and subsequent under poor storage conditions (Cheng and Hardy, 2003). Oxidized feeds adversely affect the resistance of fish against diseases, at the same time they damage both growth and development and fillet quality (Sutton et al., 2006; Grigorakis et al., 2010). Oxidation of fish oil in feed can be determined by plasma glucose, cortisol and osmolarity levels of fish and impairs the stress response (Van Anholt et al., 2004; Alves Martins et al., 2007).

It has been determined that different effects occur in fish fed with oxidized feeds. For example, in a study conducted in sea bream, it was determined that the activity of some liver enzymes with antioxidant activity such as catalase and superoxide dismutase increased. (Mourente et al., 2002). It has been observed that the immune system is negatively affected in turbot fish fed with oxidized oil feed (Obach and Laurencin, 1992). In sea bass, more fragile erythrocytes and decrease the activity of lysozyme and the complement system (Obach et al., 1993). In addition, the most common adverse effect was low nutritional quality and reduced PUFA ratios in fish fillets (Alves Martins et al., 2007; Zhong et al., 2008).

Synthetic antioxidants such as BHT, BHA and ethoxyquin have been used in fish feeds for many years in order to prevent negative and undesirable effects in fish. (Hamre et al., 2010).

The use of synthetic and natural antioxidants in aquaculture feeds has been using on for many years. However, in recent years, the use of natural antioxidants has tended to increase due to restrictions and regulations in the use of synthetic antioxidants. According to Hernandez et al. (2014), they added BHT and natural plant oil to sea bream feeds and examined the oxidation state of the feeds. According to results of the study, RO and BHT feeds showed the highest protection against induced oxidation from week 8, and at the end of the storage period, RO feed was the least oxidized.

Natural plant antioxidant sources which containing high levels of phenolic compounds have been shown to reduce oxidation as effectively as synthetic antioxidants in fish fillets (Vargas-Sanchez et al., 2019). Different natural antioxidants (ascorbic acid, tocopherol mix, rosemary, ascorbyl palmitate and etc.) were added to the fish feeds obtained entirely from raw marine products and the oxidation status of the feeds were evaluated. At the end of the study, it was concluded that the feed can be protected against oxidation by using natural antioxidants (Hamre et al., 2010).

In general, fat-soluble antioxidants such as Vitamin E (α-tocopherol) in seafood play an important role in preventing the oxidation of unsaturated fatty acids found in high amounts in fish tissues and are the groups that have the greatest antioxidant activity. Therefore, α-tocopherol acetate is a derivative of Vitamin E and is an antioxidant used to reduce oxidation of lipids in foods in general and especially in seafood (Yıldız et al., 2006).

One of the micronutrients, Vitamin C (also known as L-ascorbic acid) is a non-enzymatic antioxidant and plays an important role in reducing oxidative stress (Narra et al., 2015). Most fish cannot synthesize vitamin C and must only obtain it from external sources. Vitamin C is a powerful antioxidant because it can be oxidized and converted to less reactive substances by most of the free radicals in aqueous solution (Kefer et al., 2009; Gombart et al., 2020).

It is a natural antioxidant of vegetable origin, such as vitamin A, ß-carotene, α-tocopherol, and it makes the antioxidant effect more effective in the presence of vitamin E. However, it is known that the retinol form and ß-carotene of vitamin A are not as resistant to oxidation as vitamin E (Bai et al., 1992). Although natural antioxidants originating from vitamins are used in fish feeds, herbal extracts, marine macro and micro plankton have also been included in studies in recent years.

In recent years, it has been understood that in addition to microalgae species, macroalgae also have compounds with high antioxidant effects. The antioxidant effect contains high levels of non-enzymatic antioxidant compounds such as glutathione, ascorbic acid, α-tocopherol, β-carotene, flavonoids, hydroquinones, phycocyanins, proline, mannitol, myoinositol, phenolic compounds and polyamines (Mallick and Mohn, 2000). For example, in a study conducted on a total of 17 macroalgae species, 11 from brown algae, 1 from green algae, and 5 from red algae, it was revealed that *Sargassum spp*. had the highest antioxidant effect (Matsukawa et al., 2000). Also, Catarino et al., (2023) stated that the brown algae *Sargassum spp* is increasing in popularity day by day, especially due to its exceptional antioxidant properties.

Especially seaweed phenolics have become an attractive, sustainable source of antioxidants with a range of biofunctional properties that could potentially replace existing aquatic feed additives (Gunathilake et al., 2022).

Algae polyphenolic compounds show effective activity in delaying fish oil rancidity by acting as a good antioxidant (Mukherjee and Pal, 2021).

In a study conducted on 8 species belonging to the genus Cystoseira distributed in the Mediterranean, it was determined that these species had high antioxidant activity (Ruberto, 2001). It has been reported that algae species such as Cladophora, Chaetomorpha, Pithophora, Rhizoclonium, Spirulina, Leptolyngbya are used in feeding on goldfish and channel catfish and increase immunity as well as carotenoid content on fish (Promya et al., 2011; Mukherjee et al., 2019).

4. Conclusion

As a result, nowadays, people have started to stay away from foods containing synthetic, processed additives that are harmful to health. Likewise, as a result of the use of synthetic compounds in aquaculture, it has been revealed that there are negative effects on both fish fillet quality and human health. In recent years, it has been determined that natural antioxidants of plant origin give effective results. Therefore, it is thought that natural antioxidants will be preferred more in the future in order to prevent the oxidation of the feed used in aquaculture.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	O.T.	C.E.
C	60	40
D	60	40
S	100	
L	50	50
W	60	40
CR	60	40
SR	60	40

C=Concept, D= design, S= supervision, L= literature search, W= writing, CR= critical review, SR= submission and revision.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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